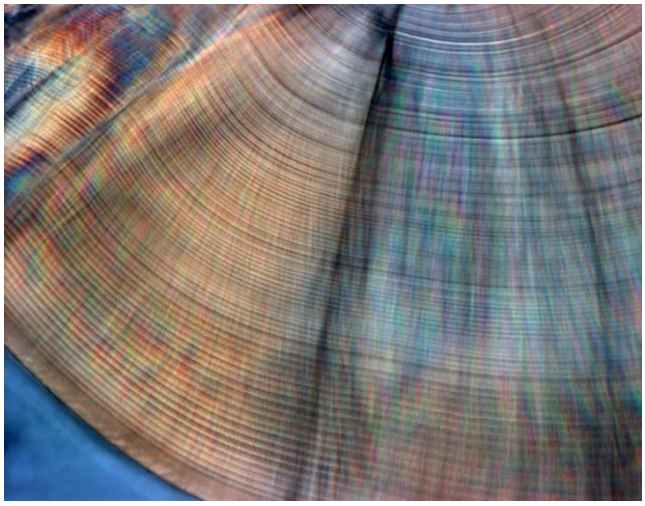
Early life history of the amphidromous galaxiid inanga: disentangling the consequences for their migratory dynamics, population structure and adult growth



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Abstract

Amphidromy is a type of diadromous migration involving pelagic larval development (mostly in the marine environment) with adult growth and maturity occurring in freshwater environments. Pelagic development and dispersal is a "black box" for many amphidromous species preventing a holistic understanding of their ecology and population dynamics. Factors driving early life history variability and relationships between pelagic and adult life are imperative to further our understanding of their demographics and population dynamics. In this thesis, I examined the life histories of inanga (*Galaxias maculatus*) within and among populations in New Zealand. Inanga are the most important component of New Zealand's culturally, recreationally and commercially important "whitebait" fishery. Populations are declining throughout the country and there is increasing pressure for tighter fishery management controls. My thesis set out to fill in some of the critical knowledge gaps pertaining to the life histories of inanga to better understand their migrations, population structure and demographics.

Studies of size, age, condition, and hatch dates of post-larvae at inward migration showed larger-scale spatial and temporal processes were driving most of the variation in the migratory characteristics of inanga. Significant differences in hatch date distributions were found among regions and were likely related to the timing of spawning within the wider area. Inanga were significantly younger and smaller at inward migration in the northern region and older and larger in the southern region. Hatch date was significantly associated with timing of inward migration, with autumn-hatched fish mostly migrating during September and winter-hatched fish during November. Although inanga were variable ages at migration, size was relatively consistent within regions showing that migration was largely size dependent.

Mixed effects modelling was used to partition the pelagic growth histories of inanga into intrinsic and extrinsic sources. Pelagic growth of inanga showed considerable variation among regions, however, growth was more consistent within regions. Seasonal variation in temperature and productivity were identified as extrinsic factors that influence growth. Inanga showed distinct developmental transitions from a pelagic-larval stages to a more competent post-larval stage. Growth during the pelagic larval stages was identified as a significant driver of age at inward migration and signifies that intrinsic growth thresholds underlie their migrations.

Current fishery management practices consider inanga as one biological unit. Otolith morphology was used to investigate the existence of putative stocks of inanga among regions with varying abiotic and oceanographic conditions. Spatial structuring of inanga was found that largely reflected the direction of the dominant ocean currents in New Zealand. Regions with high levels of mixing from other natal sources were also identified. The existence of distinct stocks shows that dispersal among some regions is restricted and inanga should be considered as multiple regional stocks for management of the fishery.

Reconstructions of the life time growth histories of adult inanga were done to investigate relationships between pelagic and adult growth. Temporal variation in abiotic conditions (as defined by hatching time) had a significant effect on growth. Winter- and spring-hatched fish with faster pelagic growth rates were slower growing for their age in rivers. On the contrary, autumn-hatched fish with slower pelagic growth rates maintained higher growth rates for their age as adults in the freshwater habitat. Age at sexual maturity and reproductive investment showed substantial variation among hatching times, however, no differences in body size at sexual maturity were found. Inanga can utilise pelagic and freshwater habitats to maximise growth and ultimately body size at maturity.

The life histories of inanga are shaped by a suite of factors acting at various spatial and temporal scales that affect their inward migrations, population structure and adult population demographics.

Chapter 1: General introduction

1.1 An overview of amphidromy

Migration is generally defined as the movement of individuals, populations, or parts of populations between two different habitat types that can be predicted on a temporal basis (Dingle and Drake 2007). Among migratory species, fish exhibit one of the most complex types of migration, diadromy, involving movement between marine and freshwater environments (Myers 1949). Three types of unique migrations are defined within diadromous fishes depending on the direction and purpose of migration (McDowall 1988). Anadromy involves migrations from marine to freshwater environments for reproduction (e.g., salmonids). The reverse is found in catadromous species (e.g., eels). Amphidromous migrations typically involve downstream larval transport, dispersal and development in a pelagic environment followed by inward migration of post-larvae to freshwater where most feeding and growth occurs (McDowall 1998).

Amphidromy is structurally and functionally different from anadromy and catadromy because of larval dispersal from natal rivers to the sea where early growth occurs. Additionally, inward migration to freshwater is initially for increased feeding followed by reproduction (McDowall 1988). However, several amphidromous species show adult downstream migration for spawning, which consequently blurs the distinction between amphidromy and catadromy (Augspurger et al. 2016).

Dispersal of amphidromous larvae from their natal freshwater habitat to a pelagic habitat seems precarious and there are potential costs associated with this type of lifecycle. McDowall (2010) postulated that:

- migration and energetics,
- navigation,
- osmoregulatory challenges,
- occupying suitable habitats,
- finding and adapting to food, and
- predation during migration

are some of the difficulties amphidromous species must overcome. Although marine larval dispersal is seemingly risky, McDowall (2010) proposed several reasons for the existence of amphidromy:

- enhanced dispersal to vacant habitat,
- enhanced colonisation of habitat from which fish had previously been eliminated by disturbance,
- predator avoidance,
- adult habitat suitability,
- adult adaptation to steep topographies in island catchments,
- maintenance of high fecundities,
- beneficial source-sink population dynamics, and
- extensive dispersal via the planktonic larval stage.

The apparent advantages of larval dispersal however do not align with the potential risks and costs incurred (Closs et al. 2013). McDowall (2010) further outlined several possible mechanisms to minimise dispersal of amphidromous species from the natal site:

- homing behaviour,
- increased larval size at emigration,
- reduced larval duration but at a cost of being smaller at migration and
- exploitation of oceanographic features.

Despite the costs and benefits of amphidromy (McDowall 2010), the evolutionary and ecological drivers of amphidromy are poorly understood (Closs et al. 2013). There is a growing body of work questioning McDowall's (2010) premise that the primary function of amphidromy is larval dispersal. Schmidt et al. (2011) proposed the "dispersal limitation hypothesis" whereby larval dispersal is constrained for amphidromous species living in hydrologically stable environments such as those of continental rivers. They state that larvae should develop in highly productive freshwater plumes or in estuaries that afford good growing opportunities and limit the risk of marine dispersal (Schmidt et al. 2011). In a comprehensive review by Augspurger et al. (2016), immense life history diversity was found across eight families (and the multiple species within) where amphidromy is prevalent. The authors categorised seven different types of life histories; fluvial, landlocked, freshwater amphidromy, marine amphidromy, catadromy, estuarine and ocean locked. They suggested that based on the considerable flexibility of their life histories, amphidromy is not necessarily a diadromous migration. Instead, amphidromy is considered a "benthic-pelagic migration" (Warburton 2015) that is driven by egg size/fecundity trade-offs (Closs et al. 2013) rather than dispersal (McDowall 2010). The rationale for this hypothesis is that, because the pelagic habitat is productive, larvae do not require large energy reserves. Consequently, their egg sizes are small.

Instead, the life history strategy of amphidromous species is to invest in high fecundity relative to non-migratory species with similar body size (Closs et al. 2013).

1.2 Amphidromous early life histories and current state of knowledge

It is evident that the theoretical framework on amphidromous life histories and their migrations remains unresolved with little consensus on what amphidromy is and why it occurs. Most of the research to date on amphidromous species has been biased to studies on a handful of species, mostly in the tropics (see Fig. A.1.1). From an ecological perspective, establishing the relationships between pelagic-larval life and adult-freshwater life is the critical link to further our understanding of amphidromous life histories, their demographics and population dynamics. Studies of amphidromous early life histories (ELH) are challenging because of the extensive larval dispersal period in the pelagic which often means larvae cannot be located or identified (Hickford and Schiel 2003). Nevertheless, a suite of tools are available to researchers to make inferences about their pelagic larval life. Two of the most popular tools used are genetic and otolith microchemistry methods.

1.2.1 Genetic methods

Genetic studies are typically used to quantify inter- and intra-population connectivity and most work has focused on the role of larval dispersal in structuring populations on evolutionary (Chubb et al. 1998, Waters et al. 2000, Carrea et al. 2013) and ecological time scales (Schmidt et al. 2011, Hughes et al. 2014). Although genetic markers show panmixia for some amphidromous species; Australian Grayling (*Prototroctes maraena*; Schmidt et al. 2011) and inanga (*Galaxias maculatus*; Waters et al. 2000), others species show genetic structuring; Australian Southern smelt (*Reteropinna* sp.; Hughes et al. 2014) and torrentfish (*Cheimarrichthys fosteri*; Warburton 2015). Even among neighbouring freshwater populations, no genetic structure was evident for adult *Sicyopterus lagocephalus* (Berrebi et al. 2005), but weak structuring was detected among four populations of adult *Galaxias maculatus* (Barker and Lambert 1988).

One problem with genetic studies is that high "potential" dispersal might be mismatched with the actual or "realised" extent of dispersal. Because only a few individuals with long dispersal distances can produce genetic homogeneity, the spatial extent of dispersal

can be exaggerated (Ward 2000). Furthermore, the absence of genetic structure cannot be used to infer a lack of structuring of population processes. Indeed, studies that couple genetics with other phenotypic characteristics, such as larval duration, highlight these discrepancies and show that dispersal is more constrained than when viewed from genetic estimates alone (Feutry et al. 2013). However, there are few comparative studies for amphidromous species and so genetic estimates of dispersal have not been compared with phenotypic characteristics like pelagic larval duration.

1.2.2 Chemical methods

Additional information about amphidromous ELHs is gained from reconstruction of an individual's pelagic phase using otoliths (Campana and Neilson 1985). One method relies on the chemical chronologies preserved in the otolith matrix that can be used as a marker for changes in habitat use (Thorrold et al. 2002). Otolith chemistry studies have shown that for some species, either freshwater or marine larval development can occur (Shen et al. 1998, David 2004, Hicks et al. 2010, Hogan et al. 2014, Schmidt et al. 2014). In other species, such as *Rhyacichthys guilberti* only marine larval development is found (Tabouret et al. 2014) indicting this species is exclusively amphidromous.

Other studies have characterised the chemical profile throughout an individual's entire pelagic life to relate their developmental trajectory with habitat transitions. Using this approach, Shiao et al. (2015) found different marine duration times between amphidromous *Rhinogobius gigas* (30-40 d marine duration) and *Stiphodon elegans (*123 d marine duration). The authors also showed that estuarine durations differed between amphidromous gobies with some spending several days in estuaries prior to inward migration, but others up to 5 months (Shiao et al. 2015). Hogan et al. (2017) used trace element chemistry along with $\delta^{18}O$ to examine larval and post-larval habitat use of *Awaous stamineus.* Their work indicated that early growth largely occurs in the near-shore low salinity environment. They also showed that individuals with a short early growth period resided in fully marine conditions, but those with a long early growth period resided in the estuary. Work by Sorensen and Hobson (2005) suggested that for O'opu Alamo'o (*Lentipes concolor),* O'opu Nopili *(Sicyopterus stimpsoni)* and O'opu Nakea (*Awaous guamensis)* in Hawaii, much of their growth is derived from the more productive inshore environments associated with freshwater plumes. They found that the chemical signature in the early to middle portion of larval life was similar to that of the freshwater plume.

Otolith microchemistry was used by Hickford and Schiel (2016) to address natal homing in a galaxiid species in New Zealand. Although some individuals returned to their natal river, the majority returned to local rivers within the wider geographic area. They also showed regional larval retention of larvae as well as some inter-regional exchange (Hickford and Schiel 2016). Altogether, these chemical methods lend important insights into the spatial ecology of amphidromous larvae and the habitats used during early life.

1.2.3 Size, age and hatch-dates

Other studies seeking to understand amphidromous ELHs have examined size and age at inward migration to freshwater. Size is often used as a proxy for pelagic growth and spatio-temporal studies are used to infer the effects of abiotic conditions on size (Radtke et al. 2001, Shen and Tzeng 2008) as well as investigate abundance at migration (Bell et al. 1995). Age at inward migration is used as a proxy for dispersal distance with older larvae considered to originate from more distant populations and younger larvae from closer populations (Lord et al. 2010, Taillebois et al. 2012). Age also reflects variation in temperature with post-larvae migrating at younger ages when pelagic conditions are warmer (Barbee et al. 2011, Teichert et al. 2016b). Size-age correlations largely show that inward migration is mostly size-dependent rather than age-dependent (Hoareau et al. 2007, Iida et al. 2008, Shen and Tzeng 2008, Lejeune et al. 2016). Back-calculated hatch dates reveal the temporal extent of spawning (Shen and Tzeng 2008) and inter-relationships with size and age show that migration timings are often (Rowe and Kelly 2009, Teichert et al. 2016b), but not always (Iida et al. 2008), synchronised with hatching times.

1.3 Using otolith-derived growth histories to understand amphidromous ELHs

Genetic and otolith-chemistry studies, along with ecological studies have proved useful for furthering our understanding of amphidromous species and their ELH. Yet, they give little insight into the mechanisms and the processes that underlie the patterns found.

Somatic growth, which is mediated by intrinsic and extrinsic factors, is recognised as the overarching factor controlling most aspects of an individual's life history (Arendt 1997). For many species, otolith size (length or width) is analogous to somatic growth in terms of fish length or weight. The internal features, or microstructure of the otolith, can be used to

approximate somatic growth during ELH (Campana and Neilson 1985). For most species, the increments are deposited at a daily resolution giving a chronology of an individual's growth history on a daily scale (Pannella 1971). Despite otolith microstructure methods being amongst the most popular techniques used in ELH studies (Starrs et al. 2016), there has been little work done on reconstructing early growth histories of amphidromous species.

Understanding growth during the pelagic phase of amphidromous species can lend myriad insights into their ELH. Studies that have explicitly reconstructed the growth histories of amphidromous species have shown that marine growth rates are correlated with sea surface temperatures (Teichert et al. 2016b). Hogan et al. (2014) showed that the ELH of O'opu nakea (*Awaous stamineus)* larvae that developed in marine and freshwater environments differed. Larvae with marine development had shorter larval durations and faster growth rates compared to larvae that developed in freshwater. For a gobiid species in the Caribbean, growth rates declined as a function of age (Lejeune et al. 2016) and similar patterns were found in other tropical species (Lord et al. 2010, Teichert et al. 2016b). Collectively, these few studies are the extent of growth reconstructions for amphidromous species using otolith microstructure methods.

1.3.1 Migration dynamics

Pelagic growth likely plays a central, yet little understood role in the inward migrations of amphidromous fish. In diadromous species, migration is typically governed by threshold traits such as body size, age or growth rate (Chapman et al. 2011). Studies have shown that faster growing fish initiate migration to the sea earlier than slow growing fish. This is the case of Atlantic salmon *Salmo salar* (Metcalfe and Thorpe 1992), sea trout *Salmo trutta* (Marco-Rius et al. 2013), and brook char *Salvelinus fontinalis* (Theriault and Dodson 2003). For diadromous species, a suite of biotic and abiotic factors as well as a genetic component underlie growth during larval and juvenile life that subsequently influences their migrations (Nichols et al. 2008, Kerr and Secor 2010). For amphidromous species, little is known about drivers of inward migration. Although local scale abiotic conditions like flood flows and temperature are thought to be important (Iida et al. 2008, Barbee et al. 2011), there is little consideration for processes occurring earlier in life that makes an individual competent to migrate. Migration competency is likely determined by growth during pelagic life, while migration itself is stimulated by biotic and abiotic factors. Consequently, understanding the drivers of growth rate variation is key to understanding the migrations of diadromous and amphidromous species alike.

1.3.2 Population structure

Many amphidromous species have a relatively wide spawning season (Keith 2003, Shen and Tzeng 2008, Hogan et al. 2014, Stevens et al. 2016) sometimes spawning multiples times within a year (Way et al. 1998, Teichert et al. 2014, Stevens et al. 2016, Teichert et al. 2016a). Coupled with a relatively high reproductive output (McDowall 1968, Closs et al. 2013, Teichert et al. 2014, Stevens et al. 2016) and geographical variation in the onset of spawning for at least some species (Iida et al. 2008, Barbee et al. 2011, Stevens et al. 2016), many larvae enter the pelagic at different times and in different places.

For species with presumed dispersive larvae, such as amphidromous fishes, adult reproductive patterns, duration of the pelagic larval stage, larval biology and recruitment dynamics are likely important components that can alter dispersal pathways and inter-population exchange (Treml et al. 2012). Protracted spawning in fish can produce 'split' cohorts of larvae with variable life histories that vary in space and time (Secor 2007). For example, alternative life history pathways (such as migratory and non-migratory) can arise between early and late spawned diadromous fish conditional on their early growth rates (Kerr and Secor 2010). In other instances, earlier hatched fish might disperse further from or stay closer to the natal habitat depending on the abiotic factors influencing their dispersal and how these vary temporally (Oresland and Andre 2008).

Biophysical models of larval dispersal (Opdal et al. 2011, Treml et al. 2015), direct observations of larvae in different water masses (Ciannelli and Bailey 2005), hatch date distributions (Marteinsdottir et al. 2000) and otolith increment width analysis (Fowler et al. 2000, Bruce et al. 2001, Brophy and King 2007) have been used to infer the dispersal trajectories of larvae based on their spawning times. Differences in increment width during specific stages of an individual's life can indicate that fish have experienced different environmental histories or provide evidence for movement into water masses of different characteristics (Sponaugle 2010), divergent dispersal pathways (Bruce et al. 2001), different life history strategies (Cowen 1991), adaptations for survival in different environmental circumstances (Shulzitski et al. 2016) or selection for growth rates (Gagliano et al. 2007). Moreover, evaluating increment widths during ELH can provide information about the origins of larvae (Shiao et al. 2001) and the potential for mixing between distinct spawning components (Brophy and King 2007) and can be used to evaluate population structure. Differences in otolith shape can be used to discern groups of fish that have grown much of their lives in different geographic regions (Aguera and Brophy 2011) and has been used to infer the

migratory routes of species (Duarte-Neto et al. 2008) as well as dispersal pathways conditional on hatching times (Libungan et al. 2015).

Such phenotypic and biophysical approaches have not been widely used for studies of dispersal, population structure and potential origins of amphidromous species. In one study, Lord et al. (2010) described the growth pattern of three amphidromous species using otolith microstructure. From their results, they speculated that the "migration routes" of endemic and widely distributed amphidromous gobies (Sicydiinae) differed based on different otolith growth rates. Lord et al. (2012) further quantified otolith morphology among populations of three amphidromous gobies. They found intra-population differences in otolith morphology and suggested that the oceanographic current systems in Vanuatu and New Caledonia likely restrict dispersal resulting in regional larval pools.

1.3.3 Consequences of early growth histories on adult dynamics

There is increasing evidence that larval experiences are linked to adult dynamics via "carry over effects" (Shima and Swearer 2010, Conroy et al. 2015) that can directly or indirectly alter various aspects of an individual's life history. For example, variable larval growth rates can directly affect the maturity schedules of fish. In Atlantic herring (*Clupea harengus*), individuals that experienced environmental conditions promoting fast larval growth matured at age 1, but those with slow larval growth matured at age 2 and 3 (Brophy and Danilowicz 2003). Because of the inter-relationships between body size and reproductive output in fish (Wootton 1998) variation in early growth histories can potentially contribute differentially to population replenishment. Indirectly, the effects of maturing at different times of the year and at different ages can affect the timing of reproduction such that resulting offspring encounter different pelagic conditions (Garvey et al. 2002). This has implications for larval growth, mortality and survival as well as their phenotypic characteristics (Morgan and Colbourne 1999).

The relationships between early larval growth and later growth in juvenile and adult stages are complex. Initial growth differences can (1) persist in surviving adults, (2) become negligible, or (3) be reversed by compensatory growth during later stages (Sponaugle et al. 2006, Hamilton 2008). Among anadromous salmonids, negative correlations between pre- and post- migratory growth are widely found (Marco-Rius et al. 2012). Trade-offs can occur between somatic growth and reproduction that may lead to negative relationships between growth and reproductive output (Arendt 1997). Little is known about the consequences of variable early life growth histories for adult growth and reproduction among amphidromous

species. To understand the factors that shape their population dynamics, a holistic approach is needed to relate an individual's early life growth history through to its later life stages to understand their demographics and population dynamics.

1.4 Aims

This thesis aims to fill critical knowledge gaps pertaining to the early life history of an amphidromous galaxiid (inanga). I focus on reconstructing somatic growth histories to investigate the migratory dynamics, population structure and relationships between early growth and adult demographics.

1.5 Thesis structure

Chapter 2. A spatio-temporal study of the inward migration characteristics of an amphidromous galaxiid.

In the first data chapter, I investigate spatial and temporal trends in post-larval characteristics (size, age, condition and hatch dates) within and among regions. This was done to provide some baseline measurements of this species' characteristics at migration.

Hypothesis

I hypothesised that the characteristics of post-larval inanga at migration would show greater variation among regions than within regions.

Chapter 3. Insights into the early life history of an amphidromous galaxiid using otolith-derived growth reconstructions.

In Chapter 3, I reconstruct the pelagic growth histories of post-larvae using otolith microstructure methods. I examine growth within and among regions and at various levels of biological organization (among individuals, hatch-months and cohorts) in a mixed effect modelling framework.

Hypothesis

I hypothesised that if larvae are spending most their early stages in different water masses then this would be reflected in the growth patterns recorded in their otoliths.

Chapter 4. Sagittal otolith shape analysis as a tool to discern population structure of an amphidromous galaxiid.

In Chapter 4, I use otolith morphology as a tool to discriminate autumn- and winter-hatched fish among regions. I examine the integrity of regional populations using multivariate analysis of their morphometric characteristics.

Hypothesis

I tested the hypothesis that spatial and temporal variation in spawning times, coupled with oceanography, structures the larval stages (Fortier and Leggett 1983). I predicted that larval dispersal of inanga would be constrained by different oceanographic and climatic processes among regions, as well as temporal variation in abiotic conditions and that this would result in regional stock structure.

Chapter 5. Disentangling the life-history of an amphidromous fish using somatic growth reconstructions from adults

In Chapter 5, I use otolith microstructure to age adult inanga and reconstruct their growth profiles from the pelagic-larval stage through to adult-freshwater growth. I then investigate relationships between different demographic metrics (size and age at sexual maturity along with reproductive output) and their growth histories.

Hypothesis

I predicted that variable growth during the pelagic phase of life has consequences for the demographics of adult populations. I hypothesised that age and size at sexual maturity as well as reproductive output would differ with growth as pelagic larvae.

Chapter 6. General Discussion.

This is one of the first bodies of work to systematically reconstruct the growth histories of an amphidromous species from their pelagic-larval life though to adult-growth in freshwaters. In this chapter, I discuss the results found in this thesis and provided new insights into the early life history of inanga. I discuss the role of marine growth for migrations, population structure and adult dynamics. I put my results into current fishery management context, provide new insights into amphidromous life histories and discuss future research directions.

1.6 Study species

In New Zealand, more than half of the freshwater fish fauna are diadromous, with most being amphidromous (McDowall 1998). There are five species of diadromous galaxiids: inanga (*Galaxias maculatus*), koaro (*G. brevipinnis*), giant kokopu (*G. argenteus*), banded kokopu (*G. fasciatus*) and shortjaw kokopu (*G. postvectis*). Inanga, the focal species for my thesis is frequently regarded as the most widespread freshwater fish species in the world (McDowall 1988). Inanga are found in Western Australia, through to Tasmania, New Zealand, Chile and as far south as the Falkland Islands (McDowall 1988). Within New Zealand, inanga are found throughout most of the mainland and surrounding islands and are only absent from central Fiordland, because the steep topography inhibits penetration inland (McDowall 1990). Adults typically inhabit lowland coastal streams that have open access to the sea although landlocked populations are also found (McDowall 1990).

1.6.1 Adult growth, maturity and reproduction

The demographics of adult inanga are poorly understood and there is little information on their age structure and growth rates in freshwater. Chapman et al. (2006) estimated the age of landlocked populations in Australia and showed that inanga can live for up to four years. From their growth model, females were 61mm at year one while males were 56 mm (Chapman et al. 2006). In their study, riverine populations could not be aged because their otoliths were optically uniform with no distinct translucent and opaque zones. In a mark-recapture study by Hill (2013), from their fitted growth model, the authors estimated that a 90 mm adult was 4 years old and is similar to the observations by Chapman et al. (2006).

Inanga are known as “adaptable opportunists” (Chapman et al. 2006), meaning they have a short life span, high reproductive effort, small body size, low offspring investment and high demographic resilience (Winemiller 2005). This type of strategy is favoured in environments that are regulated by density-independent ecological factors, subjected to increasing disturbance, decreasing predictability in spatio-temporal variability and high mortality (Winemiller 2005). Adults (Fig. 1.1) are mostly semelparous, but recently, repeated spawning or iteroparity has been documented (Stevens et al. 2016). Considerable within and between-population variability in size at sexual maturity occurs (Barbee et al. 2011, Stevens et al. 2016) which is indicative that maturity is not driven by a fixed body size. For New Zealand populations, sexually mature females (Fig. 1.1) range from 50 mm to 125 mm and males 50

mm to 105 mm (Stevens et al. 2016). Inanga maximise their fecundity via the production of many small eggs (Closs et al. 2013) with little evidence that egg size varies with female body size (McDowall 1968). Size at sexual maturity, as well as condition and gonad weight, tends to decline throughout the spawning season for both sexes (McDowall 1968, Barbee et al. 2011). Relationships between body size and fecundity are highly variable particularly in larger (> 60 mm) fish (McDowall 1968). For example, up to six fold differences in fecundity were found among females that were 80 mm length (McDowall 1968). Sexually mature males are typically smaller at maturity than females which suggests there are different life history strategies pertaining to growth and reproduction between sexes (Barbee et al. 2011, Stevens et al. 2016). Recently, Stevens et al. (2016) documented iteroparity among New Zealand populations. Spawning and subsequent survival is not size-dependent because males and females across a broad size spectrum were iteroparous (Stevens et al. 2016).

Spawning is protracted, occurring from January at the southernmost extent of their distribution in New Zealand through to July at the northern extent, with peripheral spawning also documented outside of these 'peak' spawning times (Mitchell 1991, Taylor 2002, Hicks et al. 2013). Spawning activity is linked to lunar and tidal cycles (McDowall 1988). Other cues such as day length and seasonal changes in temperature are deemed important for initiating spawning but are poorly understood (Barbee et al. 2011). Eggs, typically 1.2 mm in diameter (Benzie 1968) develop on riparian vegetation for 2-4 weeks requiring humid conditions for successful development (Hickford and Schiel 2011b). Recent work suggests there is a trade-off between yolk sac size and larval length at hatch; larger larvae have smaller yolks sacs and conversely smaller larvae have larger yolk sacs (Semmens and Swearer 2012). Eggs usually hatch upon tidal inundation although delayed hatching has been observed (Benzie 1968). The location of this species' spawning habitat at the interface of marine and freshwater environments is considered to facilitate rapid downstream transport of newly hatched larvae (Fig.1.1) that are approximately 7 mm at hatch (McDowall 1968). Inanga spawning times, like many of New Zealand's diadromous species, are not necessarily aligned with the most favourable periods for larval growth at sea (McDowall 1995). Marine development mostly occurs in late autumn and winter during colder, unproductive periods in the pelagic (McDowall 1995).

a)

b)

c)

Figure 1.1. Inanga (*G. maculatus)* **a)** sexually mature female (87 mm total length (L_t) **b)** newly hatched larvae (7 mm L_t) and **c)** post-larval stage upon inward migration (52 mm L_t).

1.6.2 Dispersal and larval development

Galaxiid larvae are found in the marine plankton year round, up to 250km offshore (McDowall et al. 1975) but are most abundant up to 6 km offshore (Hickford and Schiel 2003). No specific information on the distribution of inanga larvae exists. Earlier work derived larval growth rates by dividing post-larval size-at-migration by age-at-migration to estimate daily growth rate at sea (McDowall et al. 1994, Rowe and Kelly 2009). From these calculations, McDowall et al. (1994) suggest that marine growth does not differ among the migratory galaxiids and Rowe and Kelly (2009) showed that growth differed between single populations on the west coast of the North and South Islands. Inanga spend longer at sea relative to the other diadromous galaxiids, and post-larvae migrate at a larger body size (McDowall et al. 1994). Marine development and dispersal are poorly understood, but initial estimates suggest post-larvae are between 103-202 days old at migration (McDowall et al. 1994). Age at inward migration varies regionally. Rowe and Kelly (2009) found that fish were younger in the north (Mokau River, mean age = 135 d) compared to the south (Hokitika River, mean age = 145 d).

Of the diadromous galaxiids, inanga are predominantly amphidromous but recent research shows variation in their migratory behaviours. Studies from South America show some individuals developed exclusively in freshwater (Carrea et al. 2013, Górski et al. 2015). In New Zealand, freshwater larval development has also been found but only in very few individuals (Hicks, 2012). Other studies show larval development occurs exclusively in the marine environment and post-larvae migrate directly from the sea to freshwater with no evidence of an estuarine phase (Hicks et al. 2005). Landlocked populations are also found largely in the North Island (McDowall 1990). For Chilean populations, variation in habitat used for larval development (marine vs freshwater) is influenced by productivity, predictability and stability of the freshwater habitat (Górski et al. 2015). In the more productive rivers in the north of Chile, fish reside in freshwaters but at the southern extent larval development is exclusively marine (Górski et al. 2015). Larval otolith microchemistry studies indicate more restricted inter-regional larval dispersal. Larvae are retained locally, albeit in unstructured larval pools but rarely return to their natal river (Hickford and Schiel 2016).

1.6.3 Inward migrations

Little is known about the cues for inward migration, but migration is likely trigged by seasonal changes in water temperature and day length (Barbee et al. 2011) along with flood flows

(McDowall 1995). Olfactory cues from other migratory galaxiids help post-larvae to select a river to return to (Baker and Hicks 2003), but the vast majority of individuals do not return to their natal rivers (Hickford and Schiel 2016). Upstream migration rate is influenced by water clarity and stream flows (Allibone et al. 1999) as well as temperature (Bannon & Ling 2004).

Some studies show seasonal declines in size and age at migration, but this is not conclusive. Rowe and Kelly (2009) found no temporal differences in mean size or age in two rivers. McDowall (1968) found that seasonal patterns in size are river-dependent; the Awarua and Moeraki Rivers on the south-west coast of the South Island showed strong seasonal declines in mean size but the Taramakau River in the middle of the west coast had no seasonal patterns. Temporal declines in mean size and age have been reported in Australian (Barbee et al. 2011) and New Zealand populations (McDowall et al. 1994) at the regional scale but not within a region. In a more recent study, Yungnickel (2017) mostly showed that inanga migrating during July were larger than those in December and January but no differences were found from September to November.

1.6.4 Population structure

There is considerable variability in the mean size of inanga at inward migration among regions. Fish are on average larger at migration at the southern extent of their distribution in New Zealand compared to northern populations (McDowall 1968, Yungnickel 2017). McDowall and Eldon (1980) suggested sub-population structures may exist and differences in vertebral numbers between northern and southern populations also indicate this (McDowall 2003). However, mitochondrial DNA studies by Waters et al. (2000) showed extensive dispersal within New Zealand and no genetic structure was found. Yet, Vanhaecke et al. (2012) assessed adult populations in Chile using msatDNA techniques. They rejected panmixia because of low but significant spatial structuring and isolation-by-distance at a regional scale. They suggest that the population model of inanga is consistent with meta-population dynamics and highlight the role that oceanography and weather plays in shaping the intensity and direction of gene flow in this species. The methods used by Waters et al. (2000) do not have the resolution to detect ecologically relevant population structure which may partly explain the opposing results found by Vanhaecke et al. (2012). Barker and Lambert (1988) found spatial structuring of adult populations in the Bay of Plenty, New Zealand, using electrophoretic techniques. They concluded that high juvenile mortality resulted in subtle genetic differences among adults (Barker and Lambert 1988).

1.6.5 Stressors

During inward migration to freshwater, migratory galaxiids are extensively exploited as part of a cultural, commercial and recreational fishery. Although five species of galaxiids comprise the whitebait fishery, inanga are considered the most abundant making up to 88% of the total catch (Yungnickel 2017). Their complex life cycle and the lack of information on the early life stages has thus far prevented systematic management of this lucrative fishery (McDowall 1991). Identification of population structure is imperative to the management of the fishery (McDowall 1996) but is inconclusive to date.

Adult and spawning habitat degradation, along with barriers to migration and fishing mortality have cumulatively increased pressures on populations which are now in decline (Goodman et al. 2014). Degradation of riparian vegetation required for spawning by inanga is widely recognised as one of, perhaps the, most critical factor impacting populations (Hickford and Schiel 2011a). Climate change and its associated changes to sea surface temperature and circulation patterns has been implicated in the decline of this species in south-west Australia (Barbee et al. 2011).

1.7 Study sites

Sea surface temperatures (SST) were obtained from the National Oceanic and Atmospheric Administration's (NOAA) extended reconstruction of SST database version 3b (ERSST v.3b; http://www.ncdc.noaa.gov/data-access/marineocean-data/extended-reconstructed-sea-surface-temperature-ersst-v3b). Physical and environmental data for each river used for post-larval collections and adult collections were obtained from the river environment classification system (REC) (Ministry for the Environment 2010).

1.7.1 Regional marine environment

Four regions were used for this study, the Bay of Plenty, Golden Bay, Buller and Canterbury. These regions were chosen because they differ in their nearshore marine characteristics, most importantly temperature (Fig. 1.2), productivity and direction of the predominant ocean currents (Fig. 1.3).

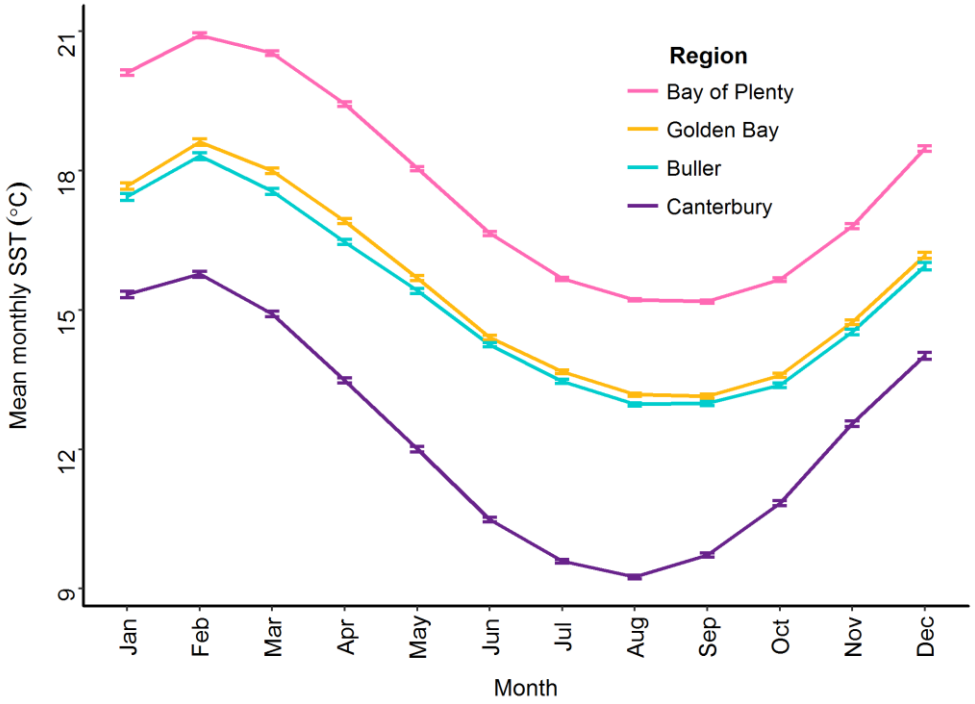


Figure 1.2. Mean monthly (± SE) sea surface temperature (ºC) in each of the studied regions.

1.7.1.1 Bay of Plenty

The climate here is warm and wet (Ministry for the Environment 2010). Surface waters in the north-east of the North Island are warm, saline and classified as subtropical (Heath 1985). Mean autumn sea surface temperature (SST) is 19.8 ºC and winter temperature is 16.4 ºC. The East Auckland current is the predominant oceanographic feature here (Fig. 1.3), originating from Australia's warm sub-tropical Tasman front and moving in a south-east direction (Chiswell et al. 2015). Upwelling systems are found along the north-east coast (Schiel 2004). The wider Bay of Plenty region is characterised by elevated chlorophyll concentrations in late winter. Productivity reaches a maximum in September and October in line with spring bloom cycles (Murphy et al. 2001). Modelling by Chiswell and Rickard (2011) indicates little exchange potential of dispersive organisms between Tauranga Harbour located in the Bay of Plenty and South Island populations. More than 90% of all propagules dispersed in a north-west direction to the top of the North Island.

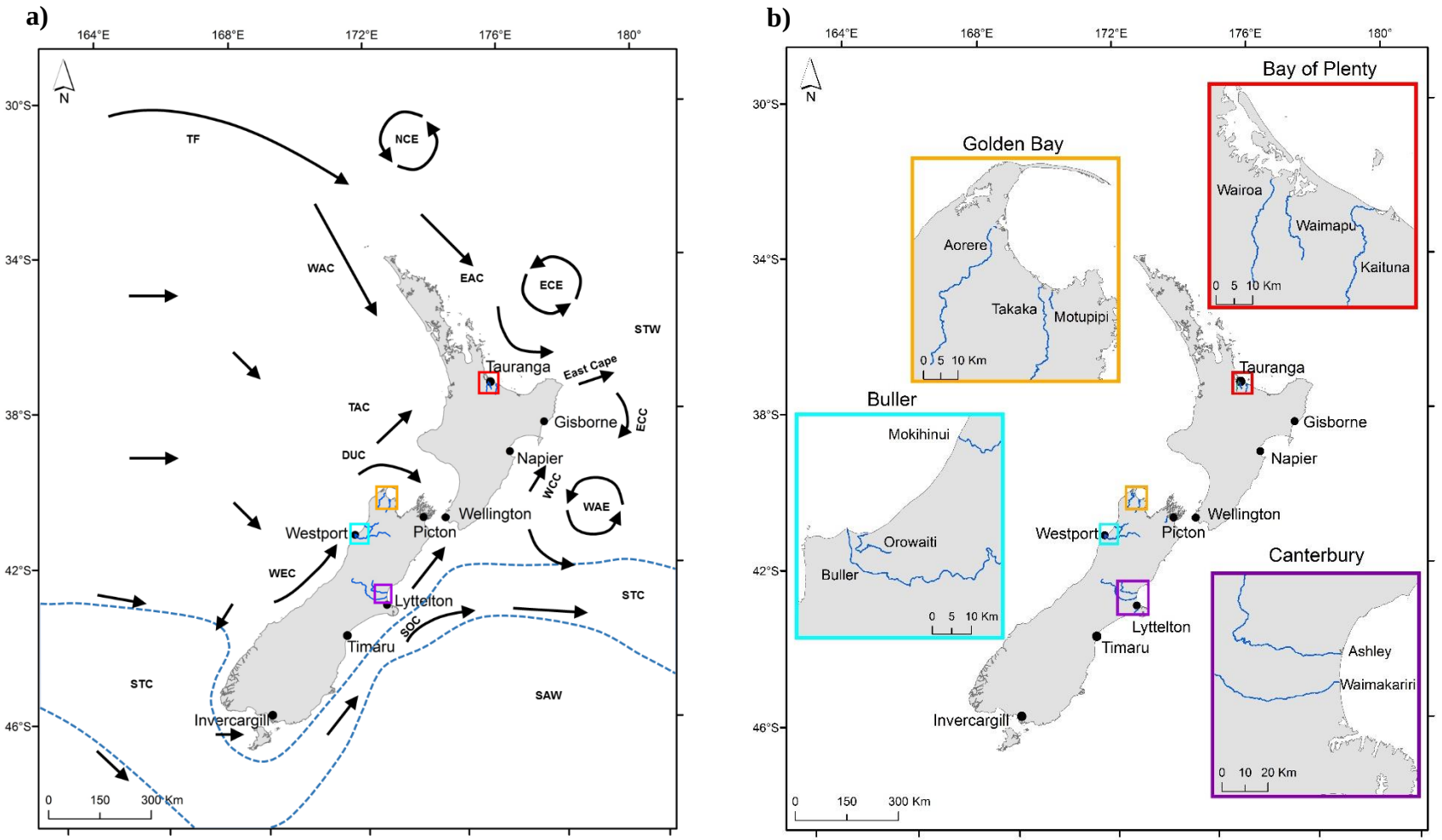


Figure 1.3. Post-larval collection sites within each of the four studied regions are shown. Panel **a)** shows the four studied regions in New Zealand, the major ocean currents and boundaries between water masses. Currents are as follows: DUC, D'Urville Current EAC, East Auckland Current; ECC, East Cape Current; ECE, East Cape Eddy; NCE, North Cape Eddy; SOC, Southland Current; SAW, Subantarctic water; STC, Subtropical Convergence; STW, Subtropical water; TAC, Tasman Current; TF, Tasman Front; WAC, West Auckland Current; WAE, Wairarapa Eddy; WCC, Wairarapa Coastal Current; WEC, Westland Current. Panel **b)** shows the rivers within each region.

1.7.1.2 Golden Bay

The current structure in the wider Golden Bay region is variable, hydrologically complex and it is considered an area of intense mixing (Chiswell et al. 2015). This region is supplied with relatively warm, nutrient rich waters from the merging of the Westland Current with the D'Urville Current in the South Taranaki Bight (Chiswell et al. 2015). The wider region is highly productive (Foster and Battaerd 1985, Bradford et al. 1986). Central upwelling systems are found at the western and eastern margins of this region and these systems, along with strong tidal forcing (Heath 1978), are suggested to inhibit the exchange of pelagic organisms from northern and southern populations (Apte and Gardner 2002). Genetic discontinuities of many species have been identified here (Ross et al. 2009) and a biogeographic break between the North and South Islands is proposed for this location (Shears et al. 2008). Satellite observations of sea-surface chlorophyll concentrations show summer upwelling off the Kahurangi Shoals (Pinkerton et al. 2013). During winter, increased precipitation and inputs of riverine/terrestrial nutrients increases nearshore productivity here (Pinkerton et al. 2013) and also further south in Tasman Bay (Gillespie et al. 2011). The climate here is cool and extremely wet (Ministry for the Environment 2010).

1.7.1.3 Buller

The Westland Current is driven by the south-westerly winds and moves along the west coast of New Zealand in a northerly direction, with weak mean flows c. 0.05 ms^{-1} (Chiswell et al. 2015). There is little evidence of seasonal variability in chlorophyll concentration in this area but a spring maximum and a winter minimum can be found (Hadfield and Sharples 1996). The Westland current periodically reverses. It alternates between weak upwelling northward flows and downwelling southward flow (Menge et al. 2003). Upwelling is intermittent and the coastal environment alternates between an upwelling phase with cold water close to shore and a downwelling phase where temperatures are more uniform (Menge et al. 2003). Inputs from terrestrial and freshwater sources are also important drivers of coastal productivity (Schiel 2004). There are many large rivers along the west coast with steep gradients and this region has a high annual rainfall, up to 2200 mm a year, that influences coastal productivity (Schiel 2004). Mean autumn SST is 16.8 ºC and winter temperatures are 14.1 ºC. Modelling by Chiswell and Rickard (2011) shows the majority of simulated propagules that arrive into Westport come from Greymouth, Milford Sound and Doubtful Sound indicating little potential

for inter-regional exchange. The climate here is classified as cool and extremely wet (Ministry for the Environment 2010).

1.7.1.4 Canterbury

The climate in Canterbury is classified as cool-dry and compared to the west coast, mean annual rainfall is much lower (c. 600 mm a year) (Schiel 2004, Ministry for the Environment 2010). This region is influenced by the Southland current, which consists of sub-Antarctic waters moving up from the southern tip of New Zealand and a narrow band of inshore sub-tropical water (Sutton 2003). The Southland current has strong northerly flows (Sutton 2003). These waters are highly variable (Schiel 2004), energetic and nutrient poor being dominated by down-welling (Menge et al. 2003). Drivers of coastal productivity here are complex and poorly resolved (Schiel 2004). Seasonal variation in chlorophyll production is subtle. It is typically lowest in the winter, and is greatest between February and March (austral late summer/autumn) (Murphy et al. 2001). The location of an eddy at the Canterbury Bight potentially retains larvae and phytoplankton (Menge et al. 2003). Mean autumn SST is 13.6 ºC and winter temperatures are 10.5 ºC. During winter, the influence of sub-tropical waters makes inshore temperatures 2 °C warmer than offshore waters (Sutton 2003). Propagule simulation studies show little exchange between Lyttelton Harbour and west coast of the South Island. The majority of propagules arriving here originate from Wellington with no propagules originating from a more southerly distribution (Chiswell and Rickard 2011).

1.7.2 Rivers within regions

1.7.2.1 Post-larval collections

For the collections of post-larvae for Chapters 2, 3 and 4, three rivers were sampled within each region. There were difficulties obtaining unpigmented fish from the Styx River in Canterbury and this reduced the number of rivers sampled in my study down to two in this region. Characteristics of rivers sampled are shown in Table 1.1. These rivers varied broadly in their characteristics.

Table 1.1. Characteristics of rivers sampled for post-larval collections in each region.

Region	Climate	Season	Mean air temperature (°C)	Mean rainfall (mm)	River	Latitude	Longitude	Catchment order	Proportion pasture	Mean annual flow (cumecs)
Bay of Plenty	Warm-wet	Summer	16.7	80.5						
		Autumn	15.5	106.4	Kaituna	-37°44'	176.41	7	0.42	38.2
		Winter	8.7	118.5	Waimapu	-37°44'	176.15	4	0.51	3.5
		Spring	14.0	84.4	Wairoa	-37°41'	176.09	6	0.24	14.6
Golden Bay	Cool-wet	Summer	16.1	79.5						
		Autumn	13.0	71.7	Aorere	-40°67'	172.66	6	0.19	26.1
		Winter	7.4	84.1	Takaka	-40°81'	172.80	5	0.13	64.3
		Spring	12.2	84.7	Motupipi	-40°83'	172.83	3	0.54	0.73
Buller	Cool-wet	Summer	14.8	183.3						
		Autumn	13.3	148.1	Buller	-41°73'	171.58	7	0.08	478.4
		Winter	9.1	184.8	Orowaiti	-41°74'	171.65	3	0.37	1.9
		Spring	11.9	190.9	Mokihinui	-41°52'	179.93	6	0.01	81.5
Canterbury	Cool-dry	Summer	15.2	44.5						
		Autumn	12.7	44.4						
		Winter	8.4	64.3	Waimakariri	-43°89'	172.70	7	0.27	156.4
		Spring	12.2	52.8	Ashley	-43°27'	172.72	7	0.25	25.4

1.7.2.2 Adult collections

For Chapter 5, adult inanga were collected from four rivers in Golden Bay (Fig. 1.4). These rivers varied in their characteristics (Table 1.2).

Figure 1.4. Location of rivers in Golden Bay used for reconstruction of adult growth histories.

Table 1.2. Characteristics of rivers within Golden Bay used for adult collections.

River	Latitude	Longitude	Catchment order	Proportion pasture
Yellow Pine	-40.58	172.67	2	0.0
Tukurua	-40.73	172.70	2	0.06
Puremahaia	-40.79	172.75	2	0.40
Motupipi	-40°83'	172.83	3	0.54

1.8 Terminology

In the amphidromous fish literature, a range of terms are used to describe amphidromous species' life stages, age, and movement from marine to freshwater environments. Similar problems were highlighted by Secor and Kerr (2009) that related to a "lexicon" of terms being used to describe variation in the migratory behaviours of diadromous fish. As research of amphidromous species progresses, it is important that the scientific community comes to a consensus on the most appropriate terminology to use, or at least make efforts to explicitly define terminology in their studies.

Barbee et al. (2011) refer to the life stage of inanga on river entry as "unpigmented juveniles", Hickford and Schiel (2016) as "whitebait", "juveniles" by McDowall et al. (1994) and Hicks et al. (2005), but Hale and Swearer (2008) use the term "larvae" referring to individuals collected in the river. In other studies of amphidromous species in New Zealand, "post-larvae" refers to the transitional stage between marine and freshwater habitats (Warburton 2015). Teichert et al. (2012) found difficulties defining life stages sampled in their study of an amphidromous goby. They explicitly define larvae as individuals from hatching to recruitment and post-larvae as individuals at freshwater entry. The authors admit these terms lack clarity and accuracy because post-larval metamorphosis occurs prior to freshwater entry. In other studies, post-larvae are identified as the developmental stages sampled in the river mouth (Shen and Tzeng 2008, Lord et al. 2010), but also refer to those sampled in the sea (Watanabe et al. 2011). Hogan et al. (2014) define post-larval stages by the presence of a metamorphic mark in the otolith from retrospective studies of adults. Throughout my thesis, I use the term 'post-larvae' to refer to unpigmented fish sampled on their inward migration from the sea to the river mouth. For the remainder of my thesis I refrain from using the term 'whitebait' unless in the context of the fishery. 'Whitebait' is a colloquial term, used throughout the world to describe life stages of many fish species and is not appropriate in the context of my work.

Another problem refers to defining the amount of time in the pelagic habitat. Pelagic larval duration (PLD) is frequently used as a measure of the amount of time an individual spends at sea (Taillebois et al. 2012, Teichert et al. 2012, Tabouret et al. 2014), but other terms include larval duration (Lord et al. 2010, Hogan et al. 2014), oceanic larval duration (Rowe and Kelly 2009, Iida et al. 2015) or age at migration (McDowall et al. 1994, McDowall and Kelly 1999). Pelagic larval duration, larval duration, and its derivatives, and age at migration

are not interchangeable terms. By definition, pelagic larval duration is the period of development spent in the water column as a plankton (Kendall et al. 2013). The true PLD of an individual can be derived from their otoliths, and check marks or abrupt changes in increment width can often signify the end of this stage and transition into a competent larval stage (Cowen 1991). Pelagic larval duration is a misleading term and throughout my thesis I use "age", incorporating the amount of time an individual spent in its pelagic larval stages, the time spent in post-larval stages as individual transitions between habitats, up until time at capture. Pelagic larval duration is used to explicitly define the length of time spent as a pelagic larva, and is derived from reconstruction of growth histories (Chapter 3).

Hale et al. (2009) use the term "settlement" to describe the movement of inanga from the pelagic water column into "benthic" freshwater habitat, Barbee et al. (2011) refer to timing of river entry as settlement whereas other studies refer to this as recruitment (Hickford and Schiel 2016) or migration (McDowall et al. 1994). 'Recruitment' appears to be the most popular term used in the amphidromous literature, but it is never defined (Shen and Tzeng 2008, Lord et al. 2010, Teichert et al. 2012). Recruitment has many meanings including recruitment to the adult population, to the fishery, to the spawning stock or to the juvenile stage. In other studies, the inward return from marine to freshwater environments is called migration (McDowall et al. 1994, McDowall 1999, Iguchi 2007, Keith et al. 2008). Throughout my thesis, I use 'inward migration' as the inward movement from marine to freshwaters. In my opinion individuals that are transitioning between habitats have not yet successfully 'recruited' to the juvenile stages and during the transition between post-larvae and juvenile stages considerable mortality likely occurs. Therefore, most individuals do not successfully 'recruit' or 'settle'.

Chapter 2: A spatio-temporal study of the inward migration characteristics of an amphidromous galaxiid

Summary

The inward migration of amphidromous species from the pelagic environment to freshwater is a critical stage in their life cycle. Disentangling the intrinsic and extrinsic drivers of inward migration is difficult because the majority of early life is spent in the vast pelagic environment. As a result, there is little understanding of the relationships between their early life history and subsequent migrations. Studies over various spatial and temporal scales can help identify the dominant processes acting on populations that influence their migrations. In this chapter, I examined the post-larval migrations of inanga (*Galaxias maculatus*) and investigated size, age and condition of post-larvae at inward migration. I explored how these relationships varied among hatch dates and month at migration as temporal proxies and among rivers and regions as spatial proxies. Results showed that processes occurring at regional scales were related to size at inward migration, and size differences among individual rivers within regions and temporally were mostly negligible. Hatch date distributions varied among regions and are likely linked with regional variation in spawning times. Age at inward migration was largely explained by hatching time, and significant differences in age were found between hatching times among regions. Post-larval condition differed among regions and month at migration. Altogether, larger scale spatial and temporal processes were driving most of the variation in the post-larval characteristics of inanga at inward migration.

2.1 Introduction

Amphidromous species have a complex life cycle involving stage-dependent habitat transitions (McDowall 2007). Spawning occurs in freshwater, followed by egg deposition and development in vegetation or benthic habitats (McDowall and Charteris 2006). Egg development time is generally short, a matter of weeks, and newly hatched larvae go to sea and develop to a post-larval stage which then migrates back to freshwater where metamorphosis and sexual maturity occur (McDowall 1988). In contrast to anadromous species (such as salmonids), for which life history models (Marschall et al. 1998), population dynamics, and their inter-relationships are reasonably well-developed (Braun and Reynolds 2014), large gaps exist for amphidromous species such as galaxiids, particularly during their critical early life

stages (McDowall et al. 1994). More often than not, studies of amphidromous species are done in single rivers (Iida et al. 2008, Lejeune et al. 2016, Teichert et al. 2016b) and numbers of fish studied are limited (Shen et al. 1998, Tabouret et al. 2014, Hogan et al. 2017). Geographic studies typically focus on genetic connectivity (Chubb et al. 1998, Waters et al. 2000) and, although insightful, are of little relevance for understanding the proximate early life histories of these species and their migrations. For amphidromous species in temperate areas, there is only a limited view of their population dynamics and a paucity of information relating to spatial and temporal differences in their early life histories and migrations. In this chapter, I examined spatial and temporal variation in size, age and condition at inward migration along with hatch dates of an amphidromous galaxiid.

Understanding spatial and temporal patterns in species characteristics and how they vary at multiple scales is key to understanding the dominant processes acting on populations of migratory fish (Gregory et al. 2017). In diadromous fish, their migrations are driven by extrinsic and intrinsic factors that vary spatially and temporally. Extrinsic abiotic factors include seasonal changes in temperature, photoperiod and flood flows while biotic factors are competition, changes in food availability and predation (Gahagan et al. 2010). Sex, age, size and physiological condition are some of the intrinsic factors related to migration (Gahagan et al. 2010, Acolas et al. 2012). In heterogeneous and dynamic environments, like the marine environment, a wide range of extrinsic conditions are likely encountered by amphidromous species during larval development that influence intrinsic factors. Consequently, substantial variation in post-larval characteristics at inward migration that are driven by spatial and temporal factors are to be expected. For example, environmental gradients in temperature, productivity and length of the growing season associated with latitude affect various aspects of diadromous life histories such as growth, size and age at migration as well as migration timing (Turner and Limburg 2012, Otero et al. 2014). At a finer resolution, differences in the abiotic characteristics of individual rivers may influence features of inward migrations such as timing (Iafrate and Oliveira 2008).

Amphidromous species have a relatively wide spawning season (Keith 2003, Shen and Tzeng 2008, Hogan et al. 2014, Stevens et al. 2016), sometimes spawning multiples times within a year (Way et al. 1998, Teichert et al. 2014, Stevens et al. 2016, Teichert et al. 2016a). Relationships between their hatch dates and characteristics at inward migration are likely a critical aspect of their life histories. This is because spawning dictates when and where larvae are released into their new environment, meaning that the conditions encountered in the pelagic environment can vary with hatching times (Simonin et al. 2016). There are tentative links

between temporal variations in abiotic conditions of the pelagic environment and the migrations of amphidromous species. For example, Shen and Tzeng (2008) suggest that autumn- and spring-hatched *Sicyopterus japonicus* experience different marine conditions during pelagic development. During spring, higher temperatures and food availability mean fish are younger, faster growing and larger upon estuarine entry. Conversely, during autumn, lower temperatures and food availability results in older, slower growing fish that are smaller upon estuarine entry. Teichert et al. (2012) showed an opposite effect of temperature on size at inward migration for *Cotylopus acutipinnis*. Summer-hatched larvae experiencing warmer temperatures were smaller and younger at migration than winter-hatched fish that experienced cooler temperatures (Teichert et al. 2012). The effects of temporal variability in abiotic conditions means that later-hatched post-larvae of *Sicydium punctatum* are faster growing for their age compared to earlier-hatched individuals (Lejeune et al. 2016).

In addition, the distribution of hatch dates over broad spatial scales can lend insights into regional fidelity and mixing of populations from different spawning components. For example, Bruce et al. (2001) and Brophy and King (2007) used hatch date distributions in conjunction with oceanographic and environmental features to make inferences about the origins, dispersal and environmental history of larvae. They showed distinct spatial structuring of larvae according to their hatch dates and suggest that ocean currents restrict inter-regional exchange of larvae hatched at different times of the year. The conditions experienced during larval life may therefore vary over the entire life time of populations dependent on hatch date and are important to identify to better understand amphidromous migrations.

2.1.1 Inward migrations of inanga

Inanga are found in freshwaters throughout New Zealand encountering sub-tropical marine waters in the north and cool-temperate waters in the south during their pelagic-larval phase. Previous studies have identified spatial variability among regions in size and age at inward migration which was tentatively linked to differences in pelagic conditions (McDowall 1968, McDowall et al. 1994, Rowe and Kelly 2009, Barbee et al. 2011). Declining size at migration throughout their main migration season was found in northern New Zealand and was attributed to younger post-larvae migrating later in the season, rather than post-larvae being slower growing (McDowall et al. 1994). Rowe and Kelly (2009) found similar results, but there was evidence of river-specific differences. Smaller post-larvae were younger at migration in the

Hokitika River on the South Island, but smaller post-larvae were slower growers and not necessarily younger in the Mokau River in the North Island.

For inward migrating inanga, there is a knowledge gap around how size, age, condition and timing of migration are related to hatch dates and how these relationships vary spatially and temporally. For inanga, considerable variability in spawning time is found within and among populations (Taylor 2002). Autumn (from March to May) is considered the peak spawning period for South Island populations (Taylor 2002, Stevens et al. 2016), whereas winter (June to August) is the peak spawning time in the northern North Island (Taylor 2002). Little spawning activity occurs on the south and east coasts of the South Island during winter (Taylor 2002), although some winter spawning has been documented recently along the west coast (Stevens et al. 2016). Given the differences in spawning times as well as oceanographic and abiotic conditions, developing larvae will undoubtedly encounter a wide range of environmental conditions depending on timing of entry to the marine environment. This may play an important role in their early life histories and subsequent migrations.

2.1.2 Aims and objectives

This study aims to investigate the characteristics of inward migrating inanga within and among regions and throughout this species seasonal migration. This was done to understand the spatial and temporal factors influencing early life histories and subsequent migration.
Specifically, I set out to:

1. Examine size at inward migration and how this varied within and among rivers within regions, and across months within regions.
2. Compare size at inward migration among regions to characterise regional trends in size.

I then examined age and hatch dates of post-larvae sub-sampled across rivers, months and regions. I set out to:

1. Establish the extent of hatching times and how this varied among regions.
2. Estimate age at inward migration and how this varied among regions and hatch dates
3. Investigate the relationship between age, hatch dates and migration date and how this varied among regions.
4. Examine body condition of post-larvae among regions, migration season and hatching times.

2.2 Materials and methods

2.2.1 Study sites and sample collections

Sampling of post-larvae at inward migration was done in 2-3 rivers in four regions (Bay of Plenty, Golden Bay, Buller and Canterbury) of New Zealand (refer to Chapter 1 for a detailed description of the study sites). Where possible, 50 post-larvae were collected twice a month for 3 months (September-November, 2013) from each river. Post-larvae were collected by a network of fishers using conventional methods (scoop nets and set nets) as they made the return migration from the sea to freshwater. Fish were collected at various locations along each river bank from multiple fishermen to help ensure a random sample was obtained. Total length (L_t, mm) and weight (g) of individuals were measured before the post-larvae were frozen at -20 ºC and transported back to the laboratory. Any post-larvae with evidence of pigmentation or with stomach contents were discarded from the samples because they were undergoing metamorphosis to the adult freshwater form. Therefore, only newly arrived 'fresh run' post-larvae were used in subsequent analysis.

2.2.2 Otolith preparation, age and hatch date estimation

A random subset of post-larvae from each sampling event and river within each region were used to estimate age and back-calculate hatch dates. To do this, sagittal otoliths were extracted, cleaned in water using a fine paint brush and mounted sulcus side up onto glass microscope slides using CrystalBond™ 509 adhesive. Sagittae were polished longitudinally using South Bay Technology aluminium oxide 12µm and 0.3µm lapping film. Where necessary, sagittae were remounted and polished sulcal side down until a complete axis from core to post-rostral edge was visible. This process was done multiple times because of the thickness of the otoliths. Polished sagittae were soaked in immersion oil for at least one week to increase clarity before photomicrographs were captured with an AxioCamHRc CCD camera attached to a Zeiss compound microscope (Axio Imager.M1). Images of the whole polished otolith were captured with a 10x objective using AxioVision Rel. 4.5 software. For the analysis of daily rings, sequences of images were taken with a 40x objective. If the right sagitta was damaged then the left sagitta was used for ageing, but in some instances both sagittae were destroyed, too cracked to accurately age, or the polishing process destroyed the visibility of rings on the outer edges. This meant an entire transect could not be identified for ageing. This reduced some sample sizes.

Estimates of age were obtained from counts of daily rings from the hatch-mark to the otolith edge using the in-built otolith app in Image Pro Premier v 9.1. The hatch-mark is well defined in inanga (McDowall et al. 1994) and was clearly identified in all otoliths used in this study (Fig. 2.1). Hatch dates were derived by subtracting age from date at capture. Hatch dates rather than "spawning dates" were estimated because daily rings before the hatch marks are less discernible (McDowall et al. 1994) and so the exact timing of egg deposition cannot be inferred. Furthermore, for fish, egg development increases with latitude (Bradbury et al. 2008) and therefore adding a fixed number of days to estimate spawning dates would introduce bias especially given the regional nature of this study along with this species' protracted spawning season.

2.3 Data analysis

2.3.1 Spatial and temporal variation in size

Size (L_t) of post-larvae at inward migration was investigated between rivers within each region across the migration season (Sep – Nov). Regional differences in size at inward migration across the migration season were then investigated. Linear mixed effects models (LMEM) were used to test for differences in L_t. First, differences between rivers within each region were tested by fitting the two-way interaction river x $month_{migration}$ for each region's dataset. Then, differences in L_t between regions were tested by pooling rivers within regions and fitting the two way interaction region x $month_{migration}$. All models were fitted with day-at-capture (DAC) as a random intercept to account for correlations among individuals sampled on the same date

2.3.2 Ageing, hatch dates and body condition

A subsample of post-larvae from each river and month were used for ageing and hatch date estimation.

2.3.2.1 Age

LMEM were used to test for differences in age within and among regions, months and hatch dates.

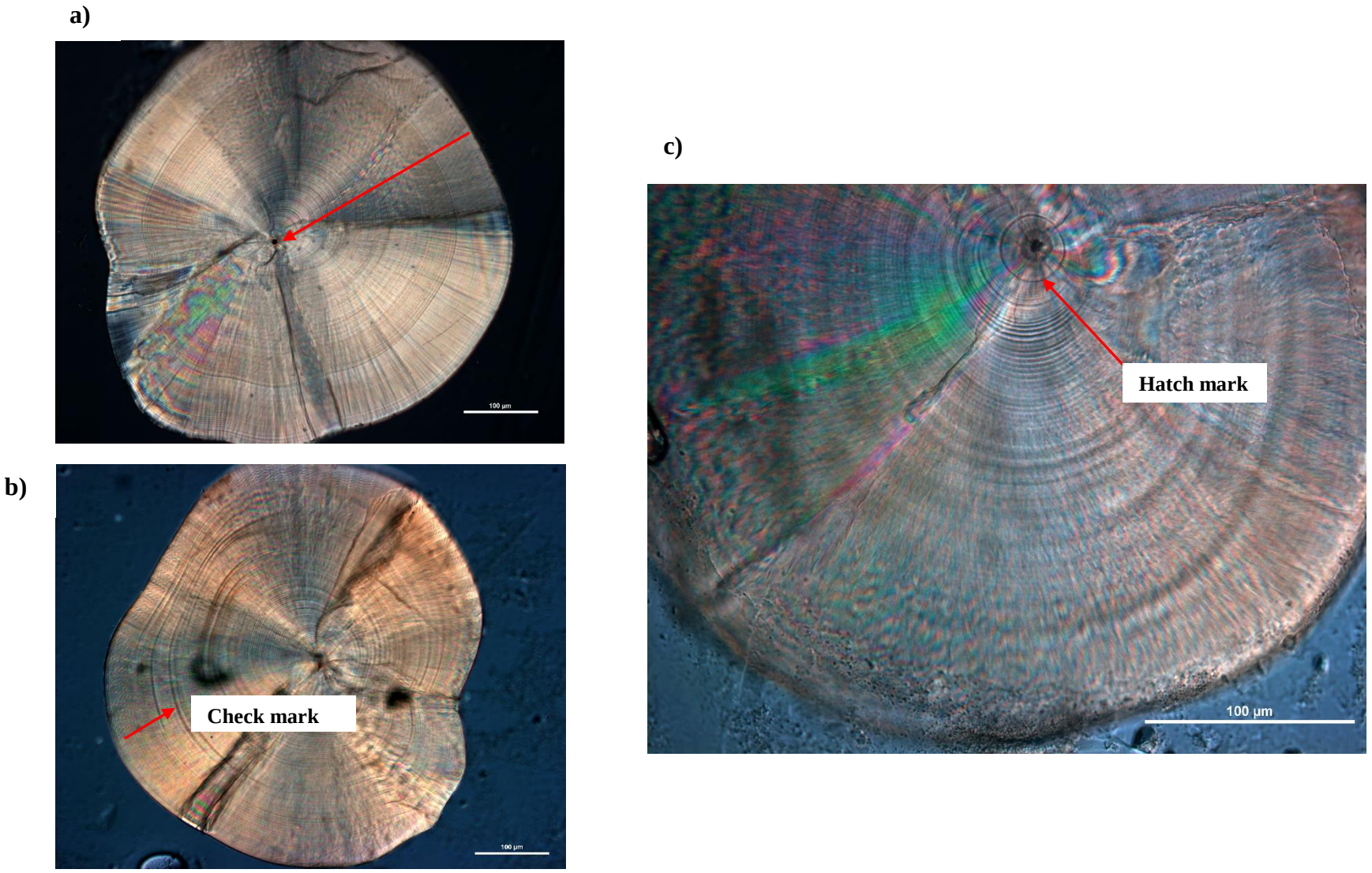


Figure 2.1. Photo-micrograph of **a)** whole otolith at 20x magnification with red arrow indicating the axis used for ageing. Panel **b)** shows whole otolith with more complex microstructure and multiple check marks evident and **c)** shows the hatch mark and daily ring deposition.

2.3.2.2 Hatch date distribution

Kolmogorov-Smirnov (KS) tests were used to test for differences in hatch date distribution within and among regions and $month_{migration}$. Spearman's correlations were used to investigate relationships between hatch date and migration-date in each region.

2.3.2.3 Size-age relationships

Relationships between L_t and age at inward migration among hatch-months across $month_{migration}$ in each region were examined using Spearman's correlations.

2.3.2.4 Body condition

The length-weight relationship for all post-larvae aged was derived using linear regression. The residuals of this relationship were used as an index of length-corrected body condition (BCI) (Heim et al. 2016). A LMEM was fitted with BCI as the response and DAC as the random term. BCI were compared among regions, month, hatch-season and their respective interactions.

2.3.3 Linear mixed effects models and statistical assumptions

For the LMEM, a top-down strategy was used whereby a model containing all interactions and main terms was fitted initially (Zuur et al. 2009). Interaction terms were then dropped sequentially and the significance of dropped terms tested using log-likelihood ratio (LR) tests. Higher level terms were removed from the model if they were not significant and only significant terms retained. LR tests were used to alleviate issues when testing between factor covariates (region, river, month) with greater than 3 levels (Zuur et al. 2009). Final models were fitted using restricted estimates of maximum likelihood (REML) to obtain unbiased parameter estimates. The proportion of variance explained by fixed effects alone ($R_2lmm_{marginal}$) and fixed effects conditional on the random effects ($R_2lmm_{conditional}$) were calculated. Multiple pairwise comparison tests (Bonferroni corrected) were used to test for significant differences in size and age within and among rivers, regions, months and hatch dates. 95% confidence intervals (CI) of the predicted values are reported.

Continuous response variables were assumed to follow a Gaussian distribution with mean and variance equals to μ and σ^2. Collinearity between predictor variables was explored so that models did not suffer from multi-collinearity. Shapiro-Wilks tests were used to test for deviation from normality and Levene's tests for variance heterogeneity. Assumptions of

statistical tests used were explored and model diagnostics (independence, variance heterogeneity, qqnorm plots) were used to validate model outputs. Response variables were not transformed as LMEM are less sensitive to non-normality than variance heterogeneity. Variance terms were included where required (Zuur et al. 2009).

All data analyses were performed in R 3.2.2 (R Core Development Team 2015). LMEM were fitted using nlme, conditional and marginal variance explained extracted using MuMIn and multiple comparisons tests were made using the multcomp package.

2.4 Results

In total, 2687 inward migrating post-larvae were collected on 42 dates between 2nd September and 20th November 2013. Although efforts were made to sample 100 individuals per river per month, this was not always achieved due to logistical constraints. There were difficulties in obtaining unpigmented post-larvae, particularly from the Motupipi River in Golden Bay. This is a highly modified system with steep banks and tannin-stained waters making it difficult for fishermen to see and capture fish which limited my sample size. Furthermore, I found higher proportions of pigmented post-larvae during November which were discarded from my samples.

2.4.1 Size at inward migration

Total length (L_t) ranged from 36.4 mm to 59.5 mm. The mean L_t of all post-larvae caught was 50.6 mm (SE = 0.08, CV = 8.2%).

2.4.1.1 Bay of Plenty

Within the Bay of Plenty, significant differences in L_t of inward migrating post-larvae were found among rivers and $month_{migration}$ (LR = 38.31, $p < 0.0001$, df = 19). Post-larvae were larger during September in the Kaituna compared to the Waimapu ($\beta = 4.0$, CI = -5.37 – -2.7) and Wairoa ($\beta = 3.2$, CI = -5.38 – -1.26) Rivers. During October, fish were larger in the Wairoa compared to the Waimapu River ($\beta = 2.3$, CI = 0.19 – 4.33). Otherwise, no significant differences were found among rivers within $month_{migration}$ or within rivers among $month_{migration}$ (Fig. 2.2). Variation in L_t of post-larvae was greatest in October for the Waimapu River (CV = 7.6%) and lowest in the Kaituna River in November (CV = 3.5%). Differences between rivers explained 11% of the variation in L_t, 32% was explained by the interaction between river and $month_{migration}$ and inclusion of the random effect for DAC increased variation explained to 60%.

2.4.1.2 Golden Bay

In Golden Bay, the interaction between river and $month_{migration}$ was not significant ($F_{4,625}$ = 2.295, p = 0.06, Fig. 2.2). Significant differences in L_t were found among rivers (LR =12.10, p < 0.01, df = 7), but not among $month_{migration}$ (LR = 1.34, p = 0.51, df = 7). Overall, post-larvae were smaller in the Takaka compared to the Aorere River (β = -.9, SE = 0.24, CI = -1.45 – -0.33). Differences among rivers explained 4% of the variation in L_t. Inclusion of the random effect for DAC increased variation explained to 34%. Total length of post-larvae was the most variable in November (CV = 4.3%) and the least in October (CV = 3.6%).

2.4.1.3 Buller

The interaction between river and $month_{migration}$ was not significant. (LR = 8.899, p = 0.063, df = 7, Fig. 2.2) and no differences in L_t among $month_{migration}$ (LR = 2.416, p = 0.298, df = 5) or rivers (LR = 4.263, p = 0.118, df = 3) were found. The random effect for DAC explained 1% of the variation in L_t.

2.4.1.4 Canterbury

The interaction between river and $month_{migration}$ was not significant (LR = 2.07, p = 0.35, df = 8, Fig. 2.2). Significant differences in L_t were found between rivers (LR = 9.17, p < 0.01, df = 7) and among $month_{migration}$ (LR = 8.48, p < 0.05, df = 6), but when the model was refitted in REML $month_{migration}$ was not significant ($F_{2,3}$ = 4.67, p = 0.11) and was therefore removed. Significant differences in L_t were found between rivers ($F_{1,406}$ = 9.77, p < 0.01) and on average, post-larvae in the Waimakariri River were smaller (β = -0.80, SE = 0.25, p < 0.001) than those in the Ashley River (β = 52.6). Differences between rivers only explained 1% of the variation in L_t. Inclusion of the random effect for DAC increased variation explained to 22%.

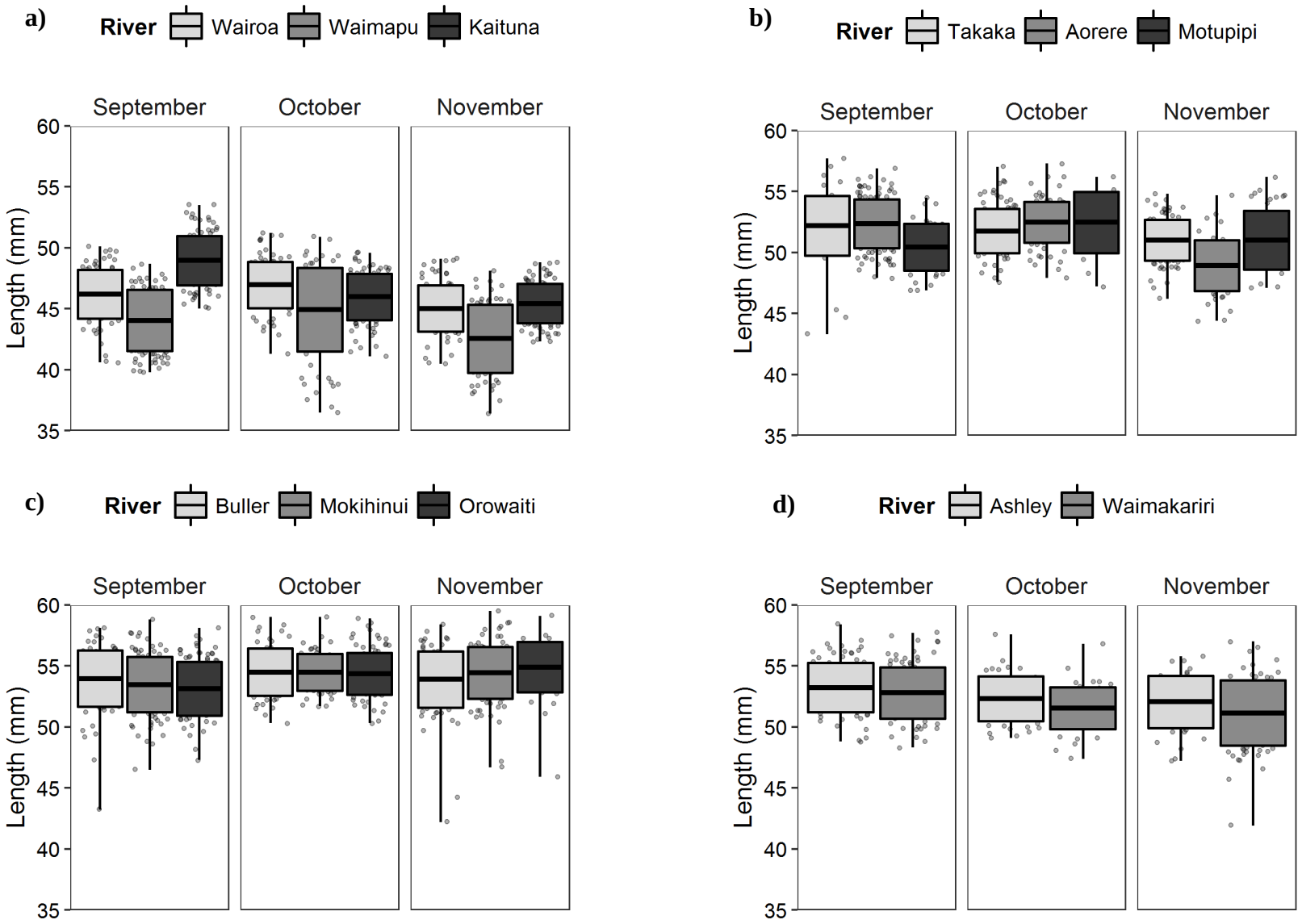


Figure 2.2. Boxplots showing mean total length of post-larvae at inward migration (mm) among rivers within regions **a)** Bay of Plenty **b)** Golden Bay **c)** Buller and **d)** Canterbury across $month_{migration}$. The height of the boxes represents the within-sample standard deviation. The lines are the maximum and minimum values observed.

2.4.1.5 Within and among regions and month$_{migration}$

The interaction between region and month$_{migration}$ was significant (LR = 39.80, $p < 0.001$, df = 19). In September, post-larvae were significantly larger in Buller (β = 7.0), Golden Bay (β = 4.3) and Canterbury (β = 7.1) than the Bay of Plenty (Fig. 2.3, Fig. A.2.1). Post-larvae in Canterbury were larger than Golden Bay during September (β = 2.7) but no differences were found between Golden Bay and Buller (β = 2.7) or Buller and Canterbury (β = 0.11, Fig. 2.3, Fig. A.2.1).

In October, post-larvae were significantly larger in Golden Bay, Buller and Canterbury (β = 5.3, β = 7.6, β = 5.5) than in the Bay of Plenty (Fig. 2.3, Fig. A.2.1). Post-larvae were significantly smaller in Canterbury compared to Buller during October (β = - 2.05, Fig. 2.3). No differences were found between Golden Bay and Buller or Canterbury and Golden Bay in October (Fig. 2.3, Fig. A.2.1). In November, greater differences in L_t were found between the Bay of Plenty and each of the South Island regions (Golden Bay; β = 6.9, Buller; β = 9.5, Canterbury; β = 6.5; Fig. 2.3). In November, post-larvae were larger in Buller compared to Golden Bay (β = 2.6) and Canterbury (β = 3.01, Fig. 2.3). No differences in L_t were found between Golden Bay and Canterbury (Fig. 2.3, Fig. A.2.1).

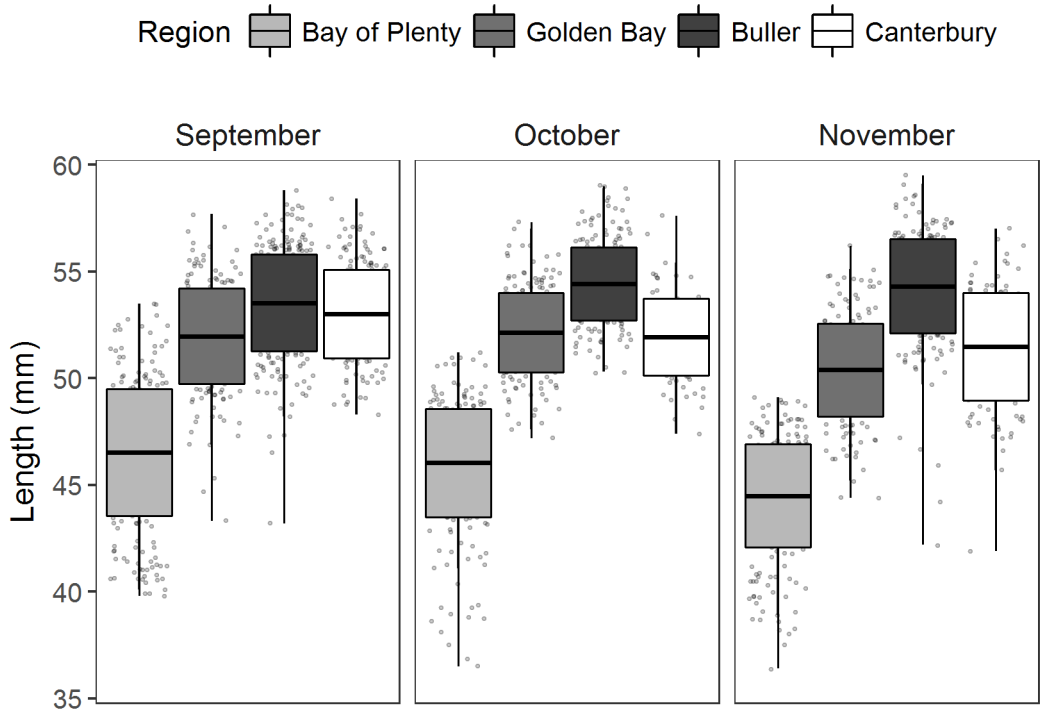


Figure 2.3. Boxplots of mean total length of post-larvae at inward migration within and among regions and month$_{migration}$. The height of the boxes is the within-sample standard deviation and the lines the within-sample range.

Within regions, no significant differences in L_t across $month_{migration}$ were found within Buller or Golden Bay (Fig. 2.3). Mean L_t of post-larvae in Buller was 54.06 mm (SE = 0.07, CV = 3.9%, n = 803) and in Golden Bay 51.54 mm (SE = 0.09, CV = 4.3%, n = 646). Post-larvae in the Bay of Plenty were significantly smaller in November compared to September (β = -2.69, CI = - 4.83 – -0.55) and October (β = -2.01, CI = -4.13 – -0.06, Fig. 2.3). Post-larvae in Canterbury were also significantly smaller in November compared to September (β = -3.32, CI = -5.78 – -0.86, Fig. 2.3). Otherwise no significant differences were found. Overall, 69% of the variation in L_t was explained by differences among regions. Including the random effect for DAC, 82% of variation in L_t was accounted for.

2.4.2 Ageing and hatch dates

For ageing and hatch date estimation, post-larvae from each river and $month_{migration}$ were pooled together within each region. This was done because most of the variation in L_t was explained among regions and $month_{migration}$ (see Section 2.4.1). In some instances, otoliths were difficult to polish or damaged during preparation resulting in variable sample sizes. The minimum number of post-larvae successfully aged per river per month in each region was 7, but in most rivers among months, more than 10 post-larvae were successfully aged (Fig. A.2.2). In total, 564 fish were successfully aged with their hatch dates estimated. At least 30 post-larvae were aged in each month in each region and they varied widely in L_t (Table 2.1).

Table 2.1. Characteristics of post-larvae that were aged in each region among $month_{migration}$.

Region	Month	n	Mean	Total length (range, mm)
Bay of Plenty	September	53	48.16	44.7 – 53.5
	October	53	45.99	38.6 – 50.7
	November	74	44.79	36.4 – 48.9
Golden Bay	September	49	51.43	44.7 – 55.8
	October	46	52.74	47.2 – 56.2
	November	30	49.14	46.2 – 52.1
Buller	September	48	53.19	48.2 – 56.7
	October	48	54.30	51.7 – 58.9
	November	47	54.70	50.8 – 58.2
Canterbury	September	42	52.64	48.8 – 57.1
	October	40	51.65	48.6 – 55.9
	November	39	51.37	45.7 – 56.2

2.4.2.1 Age at inward migration

Mean age at inward migration for all post-larvae was 124 d (n = 564, CV = 20.5%). Ages ranged from 60-187 d and varied between regions. Bay of Plenty ages ranged from 60-146 d

(mean = 95 d, n = 175, CV = 13.6%), Golden Bay from 96-176 d (129 d, 125, 13.6%), Buller from 106-182 d (139 d, 143, 11.0%) and Canterbury from 105-179 d (144 d, 121, 12.4%). Between regions, age at migration overlapped, but post-larvae less than 96 d were only found in the Bay of Plenty. Post-larvae older than 146 d were not found in the Bay of Plenty.

2.4.2.2 Hatch date distribution among regions within month$_{migration}$.

Overall, hatch dates spanned March through October. The hatch date distribution was unimodal and approximately 30% of all post-larvae hatched in June. Hatch date distributions were significantly different among regions overlapping only between Buller and Canterbury (Table A.2.1). Hatch dates in the Bay of Plenty ranged from 3 May to 23 September, but were predominantly winter- and spring–hatched fish (June to early September; Fig. 2.4). Less than 10% of Bay of Plenty post-larvae hatched in autumn. In contrast, hatch dates in Canterbury ranged from 17th March to 29th July (Fig. 2.4), but most Canterbury post-larvae hatched in April (31%) and May (29%). The distribution of hatch dates in Golden Bay was the most extensive ranging from 16th March to 13th August (Fig. 2.4). The greatest proportion of Golden Bay post-larvae hatched in May (35%), with approximately equal proportions of autumn-hatched (45%) and winter-hatched (55%) post-larvae. Hatch dates in Buller ranged from 13th March to 25th July and the greatest proportion of post-larvae hatched in June (46%).

2.4.2.3 Hatch date distribution within regions among month$_{migration.}$

Spearman's correlations showed there was a strong and significant association between hatch date and migration-date ($r_s > 0.79$, $p < 0.001$ in each region). The hatch date distribution within each region was mostly significantly different among month$_{migration}$ (Table A.2.2). In Golden Bay, Buller and Canterbury, more than 75% of post-larvae migrating in September were hatched during autumn (March – May; Fig. 2.4). In November, post-larvae hatched during winter (June – August) were more prevalent in all regions accounting for more than 80% of hatch dates in each region (Fig. 2.4). During October, a mixture of autumn- and winter-hatched post-larvae were identified in Golden Bay, Buller and Canterbury, but not in the Bay of Plenty (Fig. 2.4). The hatch dates of post-larvae in Buller overlapped during October and November (Fig. 2.4, Table A.2.2).

2.4.2.4 Size and age among hatching times

To test for differences in size and age at inward migration among hatching times, within and among regions and month$_{migration}$, post-larvae were grouped into autumn-hatched and winter-hatched (Fig. 2.5). This was done because in some cases, few post-larvae were identified in

each hatch-month category for robust analysis (Fig. A.2.3). Spring-hatched fish (n = 5) were removed as they were found only in the Bay of Plenty meaning the numbers of post-larvae in each region and $month_{migration}$ across hatch-seasons were variable (Fig. 2.5).

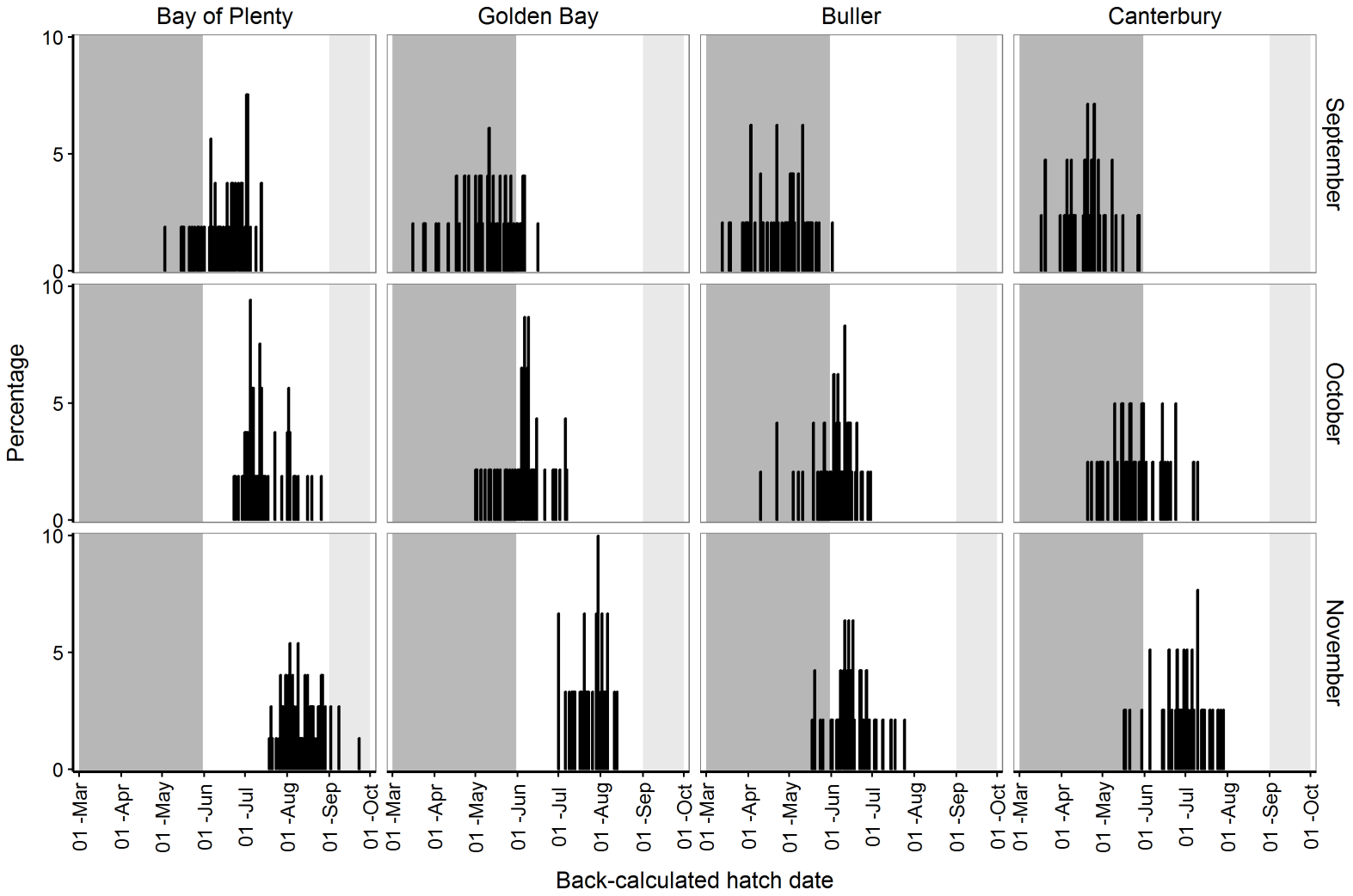


Figure 2.4. Back-calculated hatch date distribution of post-larvae expressed as a percentage per hatch date among regions and $month_{migration}$. Shaded panels denote hatch-season: autumn (dark grey), winter (white) and spring (light grey).

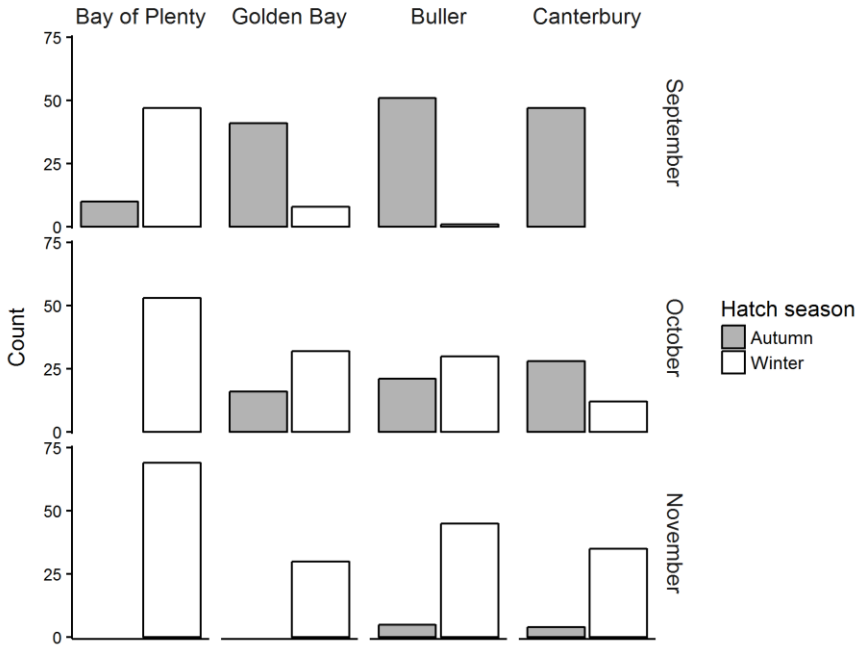


Figure 2.5. Numbers of post-larvae grouped into autumn- and winter-hatched post-larvae among regions across months.

To test for differences in L_t, two two-way interactions were fitted (region x hatch-season and $month_{migration}$ x hatch-season). The interaction region x $month_{migration}$ was not fitted because these differences were already established in Section 2.4.1. For the age model, a three-way interaction (region x hatch-season x $month_{migration}$) could not be tested because autumn- and winter-hatched post-larvae were not captured in each region in each $month_{migration}$ (Fig. 2.5). Therefore, three two-way interactions were fitted (region x hatch-season, region x $month_{migration}$ and $month_{migration}$ x hatch-season) along with the main terms.

2.4.2.4.1 Size

The interaction between region and hatch-season was significant ($F_{3,542} = 6.41$, $p < 0.001$) but the interaction between hatch-season x $month_{migration}$ was not (LR = 5.78, df = 12, p = 0.06, Fig. A.2.4). The main effects for $month_{migration}$ ($F_{2,33} = 5.23$ $p < 0.05$) and region were significant ($F_{3,542} = 166.05$, $p < 0.0001$) but no differecnes in L_t were found between hatch-seasons ($F_{1,542} = 2.17$, $p = 0.14$). Overall, 54% of the variation in L_t was explained by differences among regions. Including the random effect for DAC 79% of the variation in L_t was explained.

Autumn-hatched post-larvae were significantly larger in Buller compared to the Bay of Plenty ($\beta = 5.22$, CI = 2.48 – 7.96), Golden Bay ($\beta = 4.47$, CI = 2.25 – 6.69) and Canterbury ($\beta = 3.07$, CI = -5.17 – -0.97, Fig. 2.6). Otherwise no other significant differences were found for autumn-hatched post-larvae among regions (Fig. 2.6). For winter-hatched post-larvae, significant differences in L_t were found among all regions except Golden Bay and Canterbury

(β = 1.42, CI = -0.92 – 3.76, Fig. A.2.6). Winter-hatched post-larvae were significantly larger in Buller compared to the Bay of Plenty (β = 8.55), Golden Bay (β = 4.68) and Canterbury (β = 3.27). Post-larvae in Golden Bay (β = 3.86) and Canterbury (β = 5.28) were also significantly larger than the Bay of Plenty (Fig. 2.6, Fig. A.2.5).

No differences in L_t were found between autumn and winter-hatched post-larvae in Golden Bay (β = -0.10, CI = -1.66 – 1.46), Buller (β = 0.11, CI = -1.3 – 1.60) or Canterbury (β = -0.07, CI = -1.84 – 1.69). Winter-hatched post-larvae in the Bay of Plenty were significantly smaller than autumn-hatched post-larvae (β = -3.21, CI = -5.42 – -1.01, Fig. 2.6).

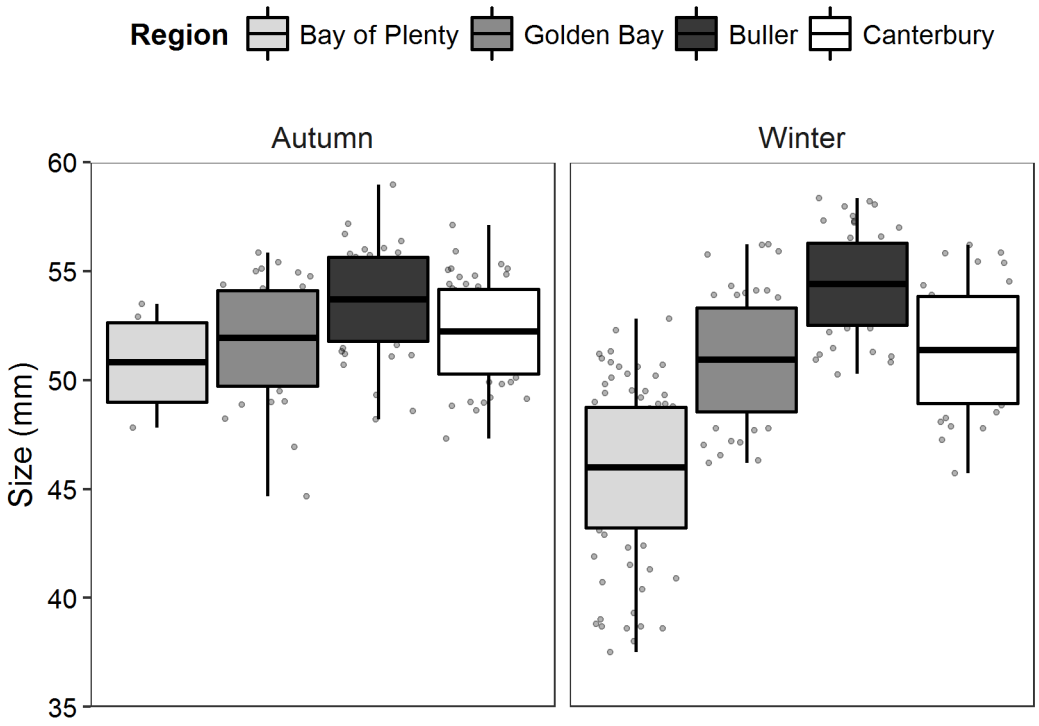


Figure 2.6. Boxplot showing mean total length of inanga post-larvae at inward migration within and among regions and hatch-seasons. The height of the boxes represents the within-sample standard deviation. The lines are the maximum and minimum values observed.

2.4.2.4.2 Age

The interactions between region and $month_{migration}$ ($F_{6,536}$ = 8.37, p < 0.001) and region and hatch-season were significant ($F_{3,536}$ = 5.43, p < 0.001). Age did not differ between hatch-seasons across $month_{migration}$ (LR = 5.67, df = 23, p = 0.06, Fig. A.2.4). Overall, 47% of variation in age was explained by hatch-season alone. Including all significant terms (and the random effect for DAC), 78% of the overall variation in age was explained.

Post-larvae from Golden Bay, Buller and Canterbury were significantly older than those in the Bay of Plenty in all months of the migration period (Fig. 2.7, Fig. A.2.6). Post-larvae in the Bay of Plenty and Canterbury showed the greatest differences in age in September, with

Canterbury being significantly older (β= 50.1, Fig. 2.7). During October, post-larvae were on average 35, 39 and 41 d older in Golden Bay, Buller and Canterbury respectively compared to the Bay of Plenty (Fig. 2.7). In November, Golden Bay post-larvae were significantly younger than those in Canterbury (β = 24.93, Fig. 2.7) and Buller (β = 20.83, Fig. 2.7), but otherwise no differences were found among regions.

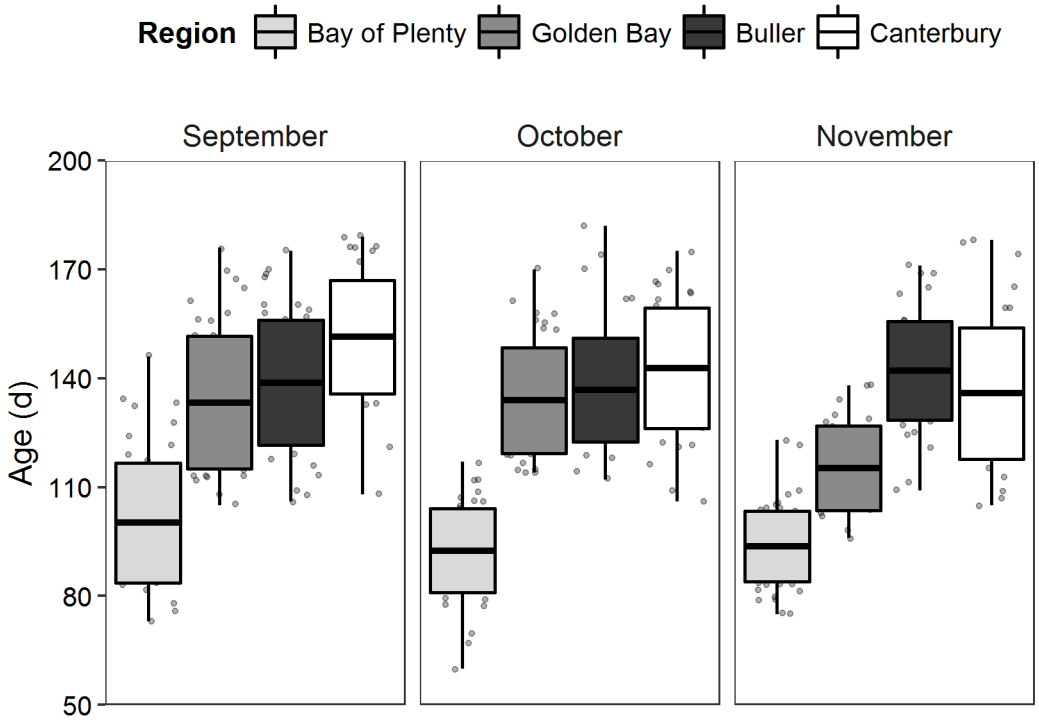


Figure 2.7. Boxplot of mean age at inward migration (d) of post-larvae within among regions and months. The height of the boxes represents the within-sample standard deviation. The lines are the maximum and minimum values observed.

No significant differences in age at inward migration were found within regions among $month_{migration}$ (Fig. 2.7). In Buller, age was relatively consistent among $month_{migration}$. Differences of only 1.8 d were found between post-larvae migrating in October relative to November (CI = -19.8 – 16.1). The greatest difference within regions was found in Golden Bay with October post-larvae on average 18.8 d older than those in November, but this was not significant (CI = -39.6 – 2.0). Post-larvae were on average 8.5 and 10.1 d younger in November than in September in the Bay of Plenty (CI = -21.9 – 7.5) and Canterbury (CI = -36.4 – 16.2) respectively but were not significantly different.

Autumn-hatched post-larvae in Buller and Canterbury were significantly older than Golden Bay (β = 18.2, β = 15.8) and the Bay of Plenty (β = 34.0, β = 31.6, Fig. 2.8, Fig. A.2.7). Age was not significantly different for autumn-hatched post-larvae between Buller and Canterbury or between Bay of Plenty and Golden Bay (Fig. 2.8). For winter-hatched post-larvae, no significant differences in age were found between Canterbury and Golden Bay (β =

5.4, CI = -5.6 – 16.5) otherwise all other pairwise comparisons were significantly different (Fig. 2.8, Fig. A.2.7). Winter-hatched post-larvae in Golden Bay were significantly older than those in the Bay of Plenty (β = 22.5) and younger than those in Buller (β = -17.4). Like autumn-hatched post-larvae, winter-hatched post-larvae in the Canterbury and Buller regions were significantly older than those in the Bay of Plenty, and the greatest difference was observed between Buller and the Bay of Plenty (β = 39.9, Fig. 2.8).

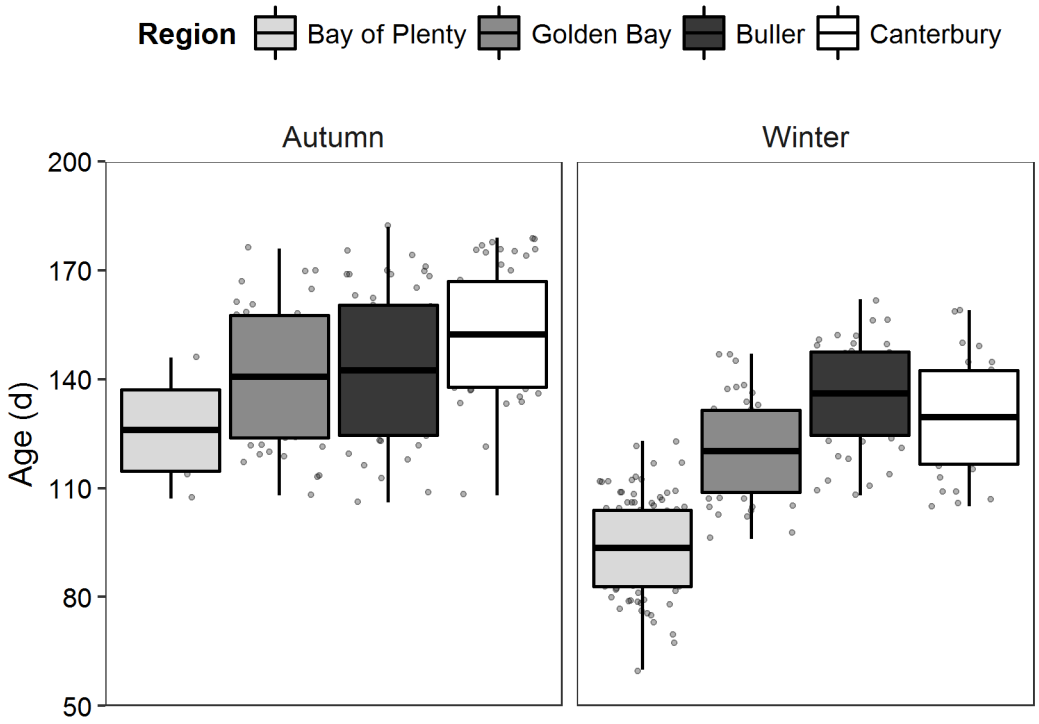


Figure 2.8. Boxplot of mean age at inward migration (d) of post-larvae within and among hatch seasons and regions. The height of the boxes represents the within-sample standard deviation. The lines are the maximum and minimum values observed.

Within regions, winter-hatched post-larvae were significantly younger than autumn-hatched post-larvae. The greatest differences observed were between autumn-hatched and winter-hatched post-larvae in Canterbury (β = -32.3, CI = -40.7 – -23.8, Fig. 2.8). In Golden Bay, winter-hatched post-larvae were 21.9 d younger than autumn-hatched post-larvae (CI = -30.4 – -13.3) and the differences between post-larvae migrating in Buller were similar (β = -22.7, CI = -30.1 – -15.2, Fig. 2.8). Winter-hatched post-larvae in the Bay of Plenty were 28.6 d younger than autumn-hatched post-larvae (CI = -44.6 – -12.6, Fig. 2.8).

2.4.3 Size-age relationships among hatch-season and month$_{migration}$

Overall, there was a strong correlation between L_t and age (r_s = 0.72, $p < 0.001$) showing that larger fish are mostly older. The strength of this association differed among regions, hatch-

season and $month_{migration}$ (Table 2.2, Fig. A.2.8). Total length and age of autumn- and winter-hatched post-larvae in Golden Bay and Canterbury during the three months were not significantly correlated (Table 2.2). Conversely, L_t and age of autumn- and winter-hatched post-larvae in the Bay of Plenty were positively correlated across all three months (Table 2.2). For post-larvae in Buller size-age relationships showed that autumn- and winter-hatched post-larvae migrating in September and October respectively, the size-age correlation was not significant (Table 2.2). In October and November size-age correlation was significant and positive for autumn- and winter-hatched post-larvae respectively (Table 2.2).

Table 2.2. Spearman's correlations between size and age within regions, hatch-seasons and $month_{migration}$. Significant correlations are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS denotes non-significant correlations. – denotes sample with less than 10 individuals and so correlations were not done.

Region	Hatch-season	September	October	November
Bay of Plenty	Autumn	**r = 0.69, ***	-	-
	Winter	**r = 0.37, ****	**r = 0.56, *****	**r = 0.29, ****
Golden Bay	Autumn	r = 0.28, NS	r = -0.18, NS	-
	Winter	-	r = 0.06, NS	r = 0.34, NS
Buller	Autumn	r = 0.09, NS	**r = 0.62, ****	-
	Winter	-	r = 0.01, NS	**r = 0.31, ***
Canterbury	Autumn	r = 0.26, NS	r = 0.30, NS	-
	Winter	-	r = -0.24, NS	r = 0.04, NS

2.4.4 Body condition

There was no difference in body condition (BCI) within and among hatch-season and $month_{migration}$ (LR = 1.42, df = 20, p = 0.49). There was no relationship with region x $month_{migration}$ (LR = 9.96, df = 14, p = 0.13) or region x hatch-season interactions either (LR = 6.14, df = 14, p = 0.10). Hatch-season did not have a significant effect on BCI (LR = 2.99, df = 10, p = 0.08). Body condition differed among regions ($F_{3,546}$ = 18.20, $p < 0.001$) and $month_{migration}$ ($F_{2,33}$ = 5.45, $p < 0.01$). Golden Bay ($\beta = 0.08$, $p < 0.007$) and Buller post-larvae ($\beta = 0.14$, $p < 0.001$) were in better condition than Bay of Plenty post-larvae (Fig. 2.9). Canterbury post-larvae had significantly lower body condition than Buller ($\beta = -0.12$, $p < 0.001$, Fig. 2.9). Overall, post-larvae that migrated in November were in better condition than those in September ($\beta = 0.12$, $p < 0.001$) and no other significant differences were found.

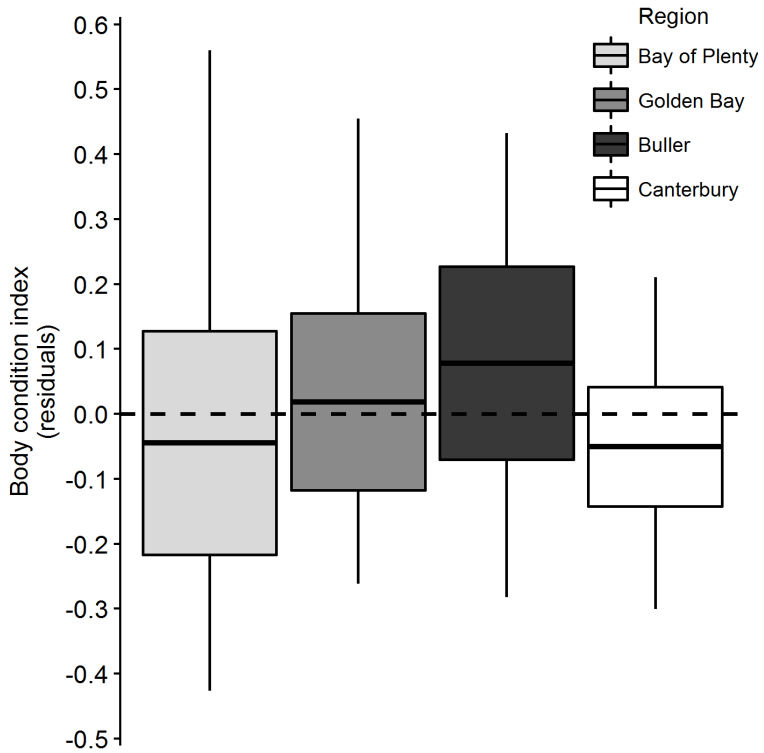


Figure 2.9. Boxplot showing mean body condition index (BCI) among regions. Lines are the maximum and minimum values observed within each sample. The height of the boxes is the within-sample standard deviation. Post-larvae in poor condition have negative values and those in good condition have positive values.

2.5 Discussion

The characteristics of migratory fish, and how they vary over multiple spatial and temporal scales are key to understanding the dominant processes acting on populations (Gregory et al. 2017). In this study, spatio-temporal variation in size, age, hatch dates and body condition of inward migrating post-larval inanga were explored. Overall, larger scale processes were identified as driving most of the variation in the characteristics of inward migrating inanga.

2.5.1 Size at inward migration

For inanga, size at inward migration was mostly consistent between rivers within regions, yet considerable regional differences were found. Similar patterns are found for other diadromous species. For example, latitude, which is a proxy for length of the growing season and climate, among other factors like habitat availability, explained 32.5% of the variation in total length of emigrating juvenile alewife (*Alosa pseudoharengus*) (Turner and Limburg 2012). Regional gradients in size are consistent with the effects of temperature on body size (Bergmann 1847, Barbee et al. 2011), but these differences might also reflect productivity gradients and growth opportunities in the pelagic environment (Gross 1987, Gross et al. 1988). For example, Ohms

et al. (2014) showed that across 16° of latitude, mean size of rainbow and steelhead trout migrating from rivers to the sea declined with increasing latitude. In northern populations, fish leave freshwater at a smaller size because growth opportunities in the sea are better than in freshwater while the opposite holds true for populations nearer the equator (Ohms et al. 2014). Such mechanisms might partly explain the regional variation in size at inward migration found in this study. Results from the body condition index (BCI) corroborate regionally variable growing conditions in the pelagic environment. Post-larvae in the Bay of Plenty were in poorer condition relative to post-larvae in Buller and Golden Bay. This suggests that the growing environment is poorer in the Bay of Plenty and may partially explain why inanga migrate inwards at smaller and younger ages. Inanga in Canterbury were in poorer condition compared to Golden Bay and Buller. The pelagic environment in the wider Canterbury region is both colder and less productive than these regions and likely impacts on post-larval condition. Little is known about the food preferences, thermal tolerance and other characteristics of inanga larvae that affect body condition. BCI is a coarse metric of an individual's energetic status and other methods like RNA:DNA analysis can give better insights into condition (Pepin et al. 1999).

Substantial variation in size at inward migration at a daily resolution was found, accounting for at least 20% of size variation in the Bay of Plenty, Golden Bay and Canterbury but not Buller. This result corroborates McDowall and Eldon's (1980) observations of considerable day- to day-variation in the size of post-larval inanga. Size may fluctuate due to finer scale changes in abiotic conditions like flow, which may impede migration on any given day leading to reduced growth (Gahagan et al. 2010), but may also be related to biotic factors like density of post-larva at migration or predation. Daily variation in size is likely an important feature of this species' migratory dynamics because it may allow for size-selective mortality processes to occur during the post-larval to juvenile transition stage (Sogard 1997). Once this daily variation was accounted for, size was mostly consistent within individual rivers among months. Furthermore, size was consistent across months in Golden Bay and Buller, but not in Canterbury (the most southern region) or the Bay of Plenty (the most northern region). At the regional scale, temporal declines in size were found among Australian (Barbee et al. 2011) and New Zealand populations (McDowall et al. 1994), but the factors underlying these patterns are not well understood. For inanga in the more northern and southern populations, declining size later in the season might be related to seasonal declines in the quality of the pelagic environment (e.g. temperature or food availability), but may also be related to precipitation and flow. The Bay of Plenty and Canterbury experience lower rainfall during spring compared to

winter whereas there is little difference between seasons in Golden Bay and Buller (see Chapter 1).

Rowe and Kelly (2009) found that the size of post-larval inanga did not vary among months and suggested that there were strong selection pressures for size at inward migration. For other diadromous species, size at migration is often remarkably uniform within populations (Iafrate and Oliveira 2008, Marco-Rius et al. 2012, Turner and Limburg 2012) further showing strong selective pressures for body size. One mechanism to explain these patterns is that there are size thresholds for migration in inanga. Such thresholds are widely found among diadromous fishes such as blueback herring (*Alosa aestivalis*) (Iafrate and Oliveira 2008) as well as amphidromous species from tropical (Keith 2003, Hoareau et al. 2007, Lord et al. 2010, Lejeune et al. 2016) and temperate environments (Shen and Tzeng 2008), but are not well known for amphidromous galaxiids.

Between rivers within Golden Bay and Canterbury, there was evidence for river-specific differences in size that suggest that physical or abiotic features of rivers in these regions affects size at inward migration. In other diadromous species, a suite of abiotic and physical features are correlated with size of emigrating fish such as watershed- and estuary-area as well as temperature and river flows (Turner and Limburg 2016). Rivers within these regions mostly differed in flow (see Chapter 1) which may influence size. The factors underlying these results remain unclear, but studies over multiple years that integrate biotic and abiotic factors might lend better insight into size differences between rivers in these regions. In the Bay of Plenty, size varied among rivers across months, but no river-specific temporal trends were identified. Similar results were found by Rowe and Kelly (2009) in a comparative study of two rivers in the North and South Islands respectively. For other amphidromous galaxiids, there is no evidence of temporal trends in size for koaro (*Galaxias brevipinnis*) and only weak evidence of temporal declines for giant kokopu (*Galaxias argenteus*) (McDowall and Eldon 1980, McDowall and Kelly 1999). McDowall (1968) suggested that temporal patterns in size at inward migration of inanga are river dependent. In this study, across the 11 rivers sampled no river-specific temporal differences were identified contrary to McDowall (1968) results. In a comprehensive study by Yungnickel (2017), there was evidence for river-specific changes in size of inanga, but the differences were mostly found between post-larvae migrating at the extremes of the migration season (July/August and December) and there was little difference over the course of the peak migration season (September to November).

2.5.2 Hatching dates varied among regions

Results from back-calculated hatch dates are in agreement with the known spawning times of this species (McDowall et al. 1975, McDowall et al. 1994, Taylor 2002, Rowe and Kelly 2009). The hatch date distribution was unimodal, showing that spawning and thereby hatching occurs almost continually over the course of the spawning season and there are no temporally distinct spawning events. Regional differences in hatch date distributions suggest there are spatially variable levels of larval dispersal among hatching components or that the temporal extent of spawning within a geographic area is wider than previously known. The presence of winter-hatched fish in Canterbury was surprising because winter spawning is not widely found here (Stevens et al. 2016). This is because winter ground frosts are thought to constrain the length of the reproductive season on the south and east coasts of the South Island (Taylor 2002) and the majority of spawning on the east coast of the South Island occurs in April (Stevens et al. 2016). In other amphidromous species like the Australian Grayling (*Prototroctes maraena*), the peak and duration of the spawning season varies inter-annually and is dependent on environmental conditions in any given year (Koster et al. 2013). In years with more favourable conditions, inanga may spawn during the winter which provides a mechanism for the presence of winter-hatched post-larvae in Canterbury.

The winter-hatched fish found in Canterbury may have originated from more northerly regions where winter-spawning is more prevalent (Taylor 2002). Dispersal simulation studies by Chiswell and Rickard (2011) showed that 56% of all propagules arriving into Lyttelton Harbour in the Canterbury region originated from Wellington Harbour, with additional sources from other North Island areas such as Gisborne, Napier as well as the top of the South Island from Picton. Although Chiswell and Rickard (2011) study provides evidence of a northerly to southerly flow of propagules to the Canterbury region, there is no specific information about the dispersal of inanga and so the potential origin(s) of winter-hatched fish is unresolved. Winter-hatched post-larvae were largely observed in the Bay of Plenty which is likely reflective of winter spawning being predominant in this region (Taylor 2002). The potential for winter-hatched larvae in the Bay of Plenty to have originated from South Island populations is unlikely due to the direction of the ocean currents which limit inter-island exchange (Chiswell and Rickard 2011). Although few autumn-hatched fish were found in the Bay of Plenty, this is likely an artefact of this study because autumn-hatched post-larvae were most likely migrating in July and August which was outside the sampling timeframe of this study.

In Golden Bay, the distribution of hatch dates was the most extensive and overlapped with the Bay of Plenty, Buller and Canterbury. Given that winter spawning is found for populations on the west-coast of the South Island, it is likely winter spawning also occurs in Golden Bay. In addition, based on the oceanographic complexity of Golden Bay, intermixing of both autumn- and winter-hatched components between North and South Island populations is feasible. Autumn- and winter-hatched fish were found in the Buller region, which corroborates gonad histological work by Stevens et al. (2016). Based on the direction of the ocean currents in Buller, inter-regional exchange is unlikely (Chiswell and Rickard 2011). Furthermore, work by Hickford and Schiel (2016) shows that a considerable proportion of larvae recruited to rivers from within the wider geographic area giving evidence for regional larval pools on this coast.

2.5.3 Age at migration

The age of inanga post-larvae at inward migration was highly variable, a pattern that is widely seen among amphidromous species. In the temperate goby *Sicyopterus japonicus*, differences of up to 80 days were observed between individuals collected on the same day from one river in Japan (Iida et al. 2008) and a cohort of the Caribbean goby *S. punctatum* differed in age by 47 days (Lejeune et al. 2016). Post-larvae less than 96 day old were only found in the Bay of Plenty and were considerably younger than the age estimates provided by McDowall et al. (1994), (range = 103 - 202 days) and Rowe and Kelly's (2009) study (range = 104 - 168 days). Regional differences in age at inward migration followed similar regional trends as size, with inanga being significantly younger at migration in the Bay of Plenty and older at migration in Canterbury. Temperature is therefore the most likely driver of variation in age at inward migration among regions (Barbee et al. 2011). Warmer sea surface temperatures experienced by larvae in the Bay of Plenty likely result in faster growth rates and coupled with shorter stage durations larvae migrate at an earlier age. Slower growth rates and longer stage durations may explain the older ages of Canterbury post-larvae.

Although age was variable, hatch-season accounted for 47% of this variation. Autumn-hatched fish were significantly older at migration than winter-hatched fish within each region, and significant differences were evident among regions. Autumn-hatched larvae spent longer in the pelagic habitat and therefore are more likely to have dispersed further than winter-hatched post-larvae. The substantial variation in ages found among hatching times further suggests that there are different pressures acting on inanga during pelagic life. Autumn and

winter-hatched fish did not differ in size at inward migration within most regions so differences in marine growth rates likely underlie age at migration (Rowe and Kelly 2009). Size-age correlations further show that migration is mostly driven by body size and not age. One hypothesis for weak size-age correlations found is that inward migration is conditional on a threshold body size (Hoareau et al. 2007, Rowe and Kelly 2009) and is discussed in Section 2.5.1. Size-age correlations for post-larvae in the Bay of Plenty showed that younger post-larvae were smaller at migration while older post-larvae were larger. This suggests that inward migration is not tightly linked with body size for inanga in the Bay of Plenty. Autumn-hatched fish migrating in October in Buller showed similar results, as did winter hatched fish migrating in November. The processes underlying these patterns are unclear.

Many studies show post-larval characteristics like size and age vary among hatch dates in a complex way. For example, in populations of *S. japonicus*, although earlier-hatched fish were older at inward migration, they attained the same body size as later-hatched fish that were younger at migration. In contrast, Teichert et al. (2012) showed that summer-hatched *Cotylopus acutipinni*s that experienced warmer pelagic conditions were smaller and younger at inward migration compared to winter-hatched fish. For temperate amphidromous gobies, Shen and Tzeng (2008) showed that spring-hatched larvae encountering warmer more productive conditions, are faster growing and larger at estuarine ingress compared to autumn-hatched fish. In a study by Teichert et al. (2016b), hatch date explained 43% of variation in size for a tropical amphidromous goby. The authors attributed declining size among hatch dates to seasonal temperature variation in the larval environment. Together, there are complex relationships between pelagic growth and hatching times within and among regions that cannot be disentangled by studies of size and age alone.

In each region, there was a strong association between hatch date and migration-date. Although this study was only done in one year, Rowe and Kelly (2009) also showed that inanga post-larvae migrating in September and November were predominantly hatched in autumn and winter respectively while October migrants were comprised of autumn- and winter-hatched fish. It is therefore apparent that the migration schedule of inanga is generally synchronised with their hatch dates although there is some flexibility in this relationship. Similar patterns are seen among amphidromous *S. japonicus,* ayu (*P. altivelis*) and *C. hangiongensis,* whereby the earliest hatched fish are the earliest migrants (Tsukamoto and Uchida 1992, Goto et al. 2014, Iida et al. 2015). Whether there is a genetic basis for migration timing in inanga is unknown.

2.6 Conclusions

This research sheds new light on the scale of spatial and temporal processes that influence the early life history and migration dynamics of an amphidromous galaxiid. Regional differences in size, age, condition and hatch dates raise important questions about the life histories of inanga over oceanographic and climatic gradients. A better understanding of how this species' spawning times vary throughout the country and among years is needed to formulate hypotheses about the origins and dispersal of post-larvae in relation to their hatching times. This would help to better disentangle the relationships between spawning and hatching times and the early life history of inanga. Together, these results suggest that there are complex relationships between pelagic growth and hatching times within and among regions that cannot be disentangled by studies of size and age alone.

Chapter 3: Insights into the early life history of an amphidromous galaxiid using otolith-derived growth reconstructions

Summary

For migratory fish, growth underlies many aspects of their life histories and is critical to understanding their ecology and population dynamics. Amphidromous species spend their larval life in the pelagic environment and so understanding growth over this critical stage of life is difficult. In this study, the growth histories of inanga were reconstructed using the chronological information stored in otoliths. Growth was examined within and among regions that differ widely in abiotic conditions to understand extrinsic sources of growth variation and how this varies spatially and temporally. Furthermore, intrinsic factors (ontogeny, $age_{migration}$) as well as variation among individuals, cohorts and hatching-times were evaluated. The growth histories of inanga showed considerable variation among regions and there was evidence that seasonal differences in temperature and productivity influence growth. Inanga showed distinct stages in growth associated with the transition from the larval to post-larval stages. Growth during larval life was a significant driver of age at inward migration. This retrospective approach lent considerable insights into the early life history of inanga. This study is the first body of work to reconstruct early growth histories of an amphidromous species and integrate various elements of their life histories into one integrative framework.

3.1 Introduction

Hjort (1914) hypothesised that growth-related processes during "critical periods" of early life drive recruitment variation in marine populations. Ever since, research on fish early life histories (ELH) has attempted to establish links between growth, mortality and survival (Johnson et al. 2014). Numerous studies have shown that faster larval growth enhances survival in the plankton; individuals that transition through their developmental stages faster minimise the time spent in these high mortality critical larval phases (Meekan and Fortier 1996, Hare and Cowen 1997, Bergenius et al. 2002). Conversely, some studies have found that slower growing fish have higher survival rates. For example, in warm, oligotrophic waters, the

energetic demands of fast growers cannot be sustained, so they suffer higher mortality relative to slow growers (Shulzitski et al. 2016). In addition to driving fluctuations in abundance, larval growth histories can have long lasting "legacy effects" that carry through to alter key population processes (Smith and Shima 2011). For example, in diadromous fishes, early growth can influence the timing of migrations (Kerr and Secor 2010, Marco-Rius et al. 2013), their propensity to migrate (Jonsson and Jonsson 2014), or the probability of early maturation (Vollestad et al. 2004).

Disentangling sources of growth variation during early life is difficult because growth integrates the effects of intrinsic and extrinsic components over an individual's lifetime (Morrongiello and Thresher 2015). Intrinsic factors refer to biological characteristics specific to an individual such as their genetic makeup, metabolic rate, ontogeny, or maternal effects (Jonsson and Jonsson 2014, Garrido et al. 2015). Extrinsic factors are the abiotic characteristics of the environment, such as temperature, food availability, hydrology or water chemistry, that can all affect growth rates (Bergenius et al. 2005, Doubleday et al. 2015). Extrinsic factors can influence growth across a hierarchy of spatial and temporal scales with growth varying across broad scale climatic (McLeod et al. 2015) and oceanographic processes (Sponaugle and Grorud-Colvert 2006), as well as finer-scale seasonal (Schismenou et al. 2016) and daily fluctuations (Wenger et al. 2016) in abiotic conditions. Studies that are done in different environments, within and among years and throughout a species' distribution can help to further understand the role of extrinsic drivers (e.g., temperature or productivity) on growth variation (Baumann et al. 2006, Doubleday et al. 2015, Vincenzi et al. 2016). An integrative approach at various levels of biological organisation (e.g., among individuals, life stages, hatch dates, cohorts, sex or life histories) can lend important insights to how and why growth varies within and among populations (Sponaugle et al. 2006, Ciotti et al. 2014), thereby providing the critical links between ELH and population dynamics.

To date, most interest in the ELH of amphidromous species has been directed towards the consequences of larval dispersal on population connectivity (Waters et al. 2000, Lord et al. 2010, Schmidt et al. 2011, Huey et al. 2014, Lagarde et al. 2016) with the role of pelagic growth on their ELH largely overlooked. Most studies that have examined spatio-temporal variation in the ELH of amphidromous species study characteristics like size and age at inward migration as well as hatch-dates to infer spawning times. Body size at inward migration if often used as an approximation for pelagic growth (Bell et al. 1995, Radtke et al. 2001, Rowe and Kelly 2009, Barbee et al. 2011, Goto et al. 2014, Iida et al. 2015). Although body size integrates an individual's growth history, it gives little insight into how an individual grows and the

proximate drivers of growth variation during the various stages of their development. For example, a large body size can be achieved more rapidly through faster growth rates (Carlson et al. 2004), but also through prolonged growth associated with slower growth rates (Angilletta et al. 2004). Furthermore, it is difficult to disentangle if size is related to characteristics prior to departure (i.e., larval size at hatch), or is mediated by abiotic conditions during migration (Freshwater et al. 2015). As such, similar sized fish can have widely different somatic growth histories. Additionally, there is often selection pressure on size at inward migration that masks variation in growth (Marco-Rius et al. 2012). Consequently, little variation in post-larval body size is found for amphidromous species, yet there is often great variation in their ages (Shen et al. 1998, Iida et al. 2008, Rowe and Kelly 2009, Lejeune et al. 2016).

3.1.1 Using otoliths to reconstruct growth histories

In instances where direct sampling of specific life stages is impossible, otolith-based techniques can be used to infer an individual's somatic growth history. A vast amount of information about an individual is recorded via daily increment deposition in the microstructure of the otolith (Pannella 1971). Daily increment counts can be used to extract demographic information such as age at time of capture as well as birth dates. Importantly, otolith size and fish size are often proportional meaning that otolith increment width approximates daily somatic growth rates (Campana and Neilson 1985). From the daily increment pattern, a chronological sequence of an individual's age-dependent somatic growth history can be derived, providing insights into developmental progression and stage durations (Campana and Neilson 1985) during the unknown pelagic phase. Existing studies describing growth patterns using otolith microstructure among amphidromous fish do not integrate various aspects of a species' ecology and biology to help identify the drivers of variation in growth patterns (Lord et al. 2010, Hogan et al. 2014, Lejeune et al. 2016, Teichert et al. 2016, Hogan et al. 2017).

3.1.2 Early life history of inanga

The ELH of inanga (*Galaxias maculatus*) is likely complex and variable. This is partly due to their extensive distribution in New Zealand that spans 12º in latitude (McDowall 1968). New Zealand's oceanography is dynamic, with distinct temperature and productivity gradients associated with latitude, strong seasonal variation in abiotic conditions and complex ocean current systems (Chiswell et al. 2015). Spatial and temporal variation in body size and age at inward migration are tentatively linked with temperature (McDowall et al. 1994, Rowe and

Kelly 2009, Barbee et al. 2011). Furthermore, their inward migration to freshwaters are triggered by seasonal changes in water temperature and day length (Barbee et al. 2011) along with flood flows (McDowall 1995). Collectively, spatial and temporal differences in environmental factors could be important for disentangling extrinsic sources of growth variation.

Spatial and temporal differences in their spawning times are found throughout their distribution in New Zealand. Spawning occurs almost year round from late austral-summer at their southern extent, through to early spring in the North Island (Taylor 2002, Hicks et al. 2013) and, as such, larvae likely experience a wide range of abiotic conditions depending on when and where they enter their pelagic phase. For inanga, the relationship between larval/post-larval growth and hatching time is unknown. Furthermore, there is anecdotal evidence that the life-histories of early- and late-spawned fish differ. McDowall et al. (1994) and Rowe and Kelly (2009) suggest that the hydrological cue for larval hatching is tidal early in the season but related to flood flows later in the season. Variation in the hydrological cues associated with spawning times might play a part in larval growth via transport to different larval rearing habitats (e.g., offshore vs. coastal).

Hickford and Schiel (2016) suggest post-larvae on the West Coast of the South Island show some regional self-recruitment. They suggest there is likely more inter-regional dispersal on the east coast of the South Island facilitated by the speed and complexity of the Southland Current system (Hickford and Schiel 2016). Furthermore, results in Chapter 2 show regional, but also some river-specific differences in size and age at inward migration suggesting regional and/or local recruitment. If larvae are spending most of their early life in water masses with different abiotic conditions like food and temperature, then this will result in variable larval somatic growth rates. Local differences among individual rivers within regions may also impact on growth.

3.1.3 Aims and objectives

The aim of this study was to reconstruct the somatic growth histories of inanga using otolith microstructure techniques. This was done to obtain more information about the intrinsic and extrinsic sources of growth variation and to better understand their pelagic growth histories. Specifically, I was interested in understanding the role of intrinsic and extrinsic sources of growth variation and how these varied within and among regions. I aimed to:

3.1.3.1 Intrinsic

1. Describe the age-dependent somatic growth trajectory of inanga and identify specific developmental transitions.
2. Evaluate relationships between somatic growth and age at inward migration.
3. Investigate if variation among individuals and hatch-months were significant sources of growth variation and how this varied within and among regions.

3.1.3.2 Extrinsic

4. Use a temporally resolved "environmental proxy" to understand inter-annual/seasonal growth variation within and among regions.
5. Investigate if growth varies among cohorts migrating from September through to November within and among regions.
6. Examine if growth varies among rivers within regions to investigate growth variation at smaller spatial scales.

3.2 Materials and methods

3.2.1 Study sites

Sampling of inanga post-larvae at inward migration was done in two to three rivers in four regions (Bay of Plenty, Golden Bay, Buller and Canterbury). A map of the study site (Fig. 1.3) and detailed descriptions are given in Chapter 1. In brief, mean annual sea surface temperature (SST) varies from 17.7 °C in the Bay of Plenty to 12.3 °C in Canterbury with a seasonal decline to a winter minimum in each region (Fig. 1.2). Primary production in coastal waters around New Zealand varies seasonally. Spring peaks occur in the Bay of Plenty and Buller, but only slight seasonal changes are found in Canterbury (Murphy et al. 2001). Seasonal productivity in Golden Bay is poorly resolved, but further south in Tasman Bay winter and spring peaks are found (Gillespie et al. 2011).

3.2.2 Data collection

Where possible, 50 post-larvae were collected twice monthly over three months (September-November, 2013) from two/three rivers in each of the four regions. For this study, I assumed inanga completed their larval development in the marine environment. This assumption was made because post-larvae were collected at river mouths directly upon inward migration from

pelagic habitat thereby limiting the probability of freshwater residency. Furthermore, an otolith microchemistry study by Hicks et al. (2010) showed that an overwhelming majority of inanga have a marine larval phase despite open access to a lacustrine pelagic habitat. Hicks et al. (2005) further confirm marine larval development among New Zealand populations.

Total length (mm) and weight (g) of individuals were measured before the post-larvae were frozen at -20 ºC. Any post-larvae with evidence of pigmentation or with stomach contents were discarded from the samples because they were undergoing metamorphosis to the adult freshwater form. Therefore, only newly arrived 'fresh run' post-larvae were used in subsequent analysis (n = 26).

3.2.2.1 Otolith preparation

A random subset of fish sampled from each two-weekly sampling event were used for growth reconstructions from otoliths. Otoliths were prepared as in Chapter 2 (Section 2.2.2). Daily ring deposition for inanga has been validated by McDowall et al. (1994) and was assumed in this study. Estimates of age at migration (counts of daily rings) and back-calculated hatch dates were obtained in Chapter 2. Daily increment widths were measured on a linear axis from the hatch mark to the post-rostral edge using the in-built otolith app in Image Pro Premier v 9.1. Initial results showed this axis had the strongest relationship with fish length therefore giving better approximations of somatic growth. Additionally, increment deposition from the hatch mark was naturally directed towards the post-rostral axis and the presence of confounding checkmarks and convergent/divergent rings were less apparent here (Fig. 3.1). The relationship between fish size (total length (mm)) and otolith size (length (µm)) was derived using linear regression. This was done to meet the assumption that otolith growth is linear and proportional to somatic growth (Campana and Neilson 1985).

3.2.3 Growth reconstruction

Age-dependent somatic growth trajectories were reconstructed from otolith increment distances to estimate rates of change in growth throughout pelagic development. Daily incremental distances were averaged over 10 day (d) intervals. These 10 d averages were used as the response variable in the statistical analysis. This was done to 1) account for some of the measurement error inherent in otolith increment studies and 2) to reduce noise in the data. Campana and Neilson (1985) further suggest that caution must be exercised when using increment widths to infer instantaneous growth rates and that averaging growth rates over several days can account for some of this uncertainty.

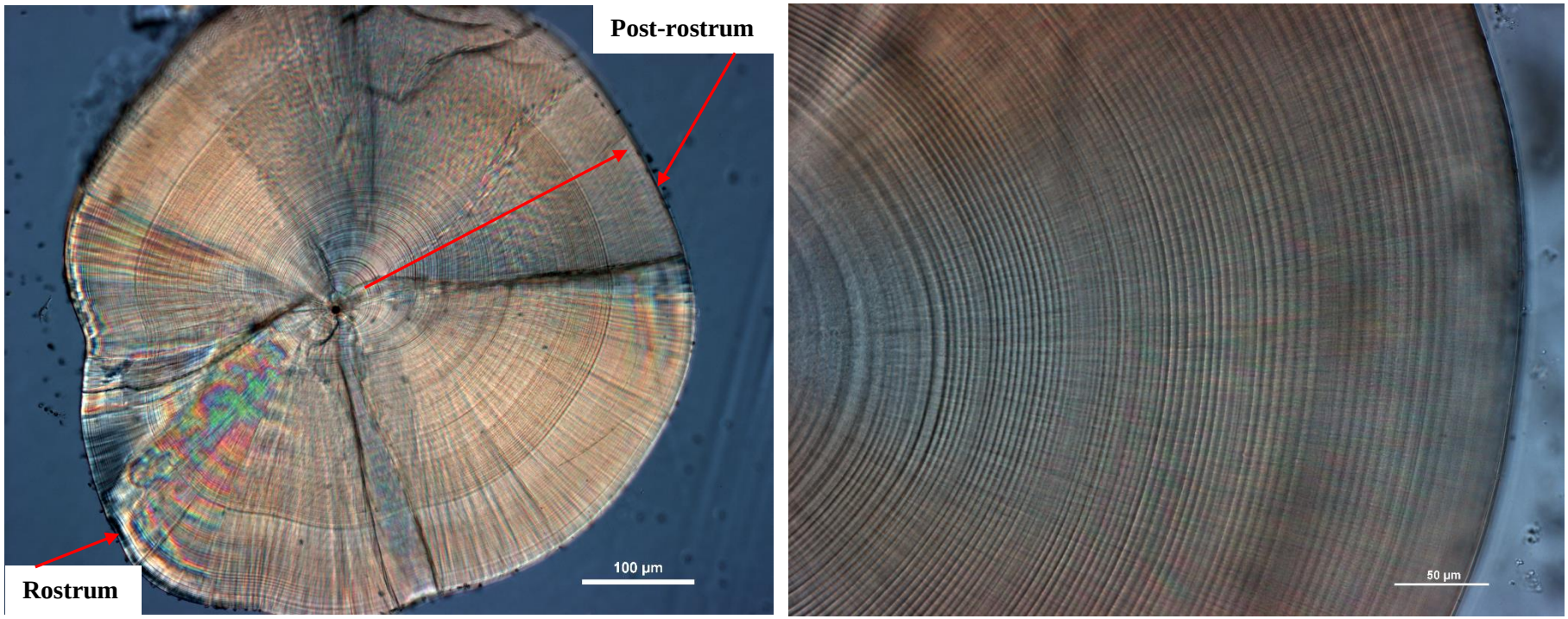


Figure 3.1. Photo-micrograph of **a)** whole otolith at 20x magnification with red arrow indicating the axis used for growth reconstruction and **b)** otolith edge at 63x magnification clearly illustrating daily ring deposition.

3.2.3.1 Assigning growth increments to "environmental proxies"

Each growth increment was assigned a date and then a month at formation. This was done to derive a temporally resolved "environmental proxy" for growth (Morrongiello and Thresher 2015). This approach is useful when the specific environmental conditions experienced by an individual are unknown. Instead, the likely environmental history can be inferred because higher or lower growth during particular times of the year can be coupled with what is known about seasonal variation in environmental conditions (Folkvord et al. 2016). The calendar day at formation for the first increment is an individual's hatch date (back-calculated) plus 1 d. The calendar day when the tenth increment was formed is an individual's hatch date plus 10 d. I then calculated the average day at formation of increments over 10 d intervals. The numbers of observations for each month in each region are shown in Fig. A.3.1.

3.3 Data analysis

Linear mixed effects modelling (LMEM) was used to partition growth among multiple sources, but also to account for some of the statistical issues with otolith increment width data (Weisberg et al. 2010). Otolith increment widths are inherently spatially and temporally auto-correlated, unbalanced and non-independent. LMEM can account for some of these issues by specifying an appropriate random effects structure (Zuur et al. 2009).

3.3.1 Predictor variables

A series of predictor variables were used to model growth (Table 3.1). Ontogenetic factors ($Age_{increment}$ and $Age_{migration}$) were modelled as fixed effects because these are the primary factors that influence growth in fish (Morrongiello and Thresher 2015). $Age_{increment}$ accounts for changes in growth with age over the course of pelagic development and $Age_{migration}$ examines how growth varies among fish that were different ages at inward migration. For each region's dataset, 'River' was treated as a fixed effect. This was done because LMEM requires greater than five levels (i.e., five rivers) in the random effects structure (Zuur et al. 2009). Random effects account for additional sources of growth variation. In this case, intrinsic (individual fish, hatch-months) and extrinsic (cohorts and temporal environmental proxies) were used (Table 3.1) because these factors were considered as a random sample from all possible fish, hatch-months and cohorts, and account for variation over the course of the growing season (temporal environmental proxies).

The random effect hatch-month refers to groups of larvae that hatched in the same month of the year and incorporates inherent differences in growth associated with timing of reproduction and thereby hatching as well as temporal variation in the pelagic environment. A cohort is defined as a group of fish that migrated in a two-week window throughout September-November. Cohorts were grouped into early (e) or late (l) for each month at migration. Cohort accounts for variation due to hatch-month, but also defines the time of the year when inanga migrate inward. Although some fish were hatched in the same month, they do not necessarily migrate at the same time (see Chapter 2), so this approach accounts for differences in migration timing, and thereby potential differences in growth. Numbers of fish in each cohort category among regions are shown in Fig. A.3.2.

Table 3.1. Intrinsic and extrinsic fixed and random effects considered in modelling somatic growth within and across regions.

		Fixed	**Random**
Intrinsic	*Ontogenetic*	$Age_{increment}$ (days, continuous) $Age_{migration}$ (days, continuous)	$Age_{increment}$ (age slope, continuous)
			Fish_ID (individual fish, factor)
			$Hatch_{month}$ (factor)
Extrinsic	*Spatial*	River (factor)	Region (factor)
	Temporal		Cohort (factor)
			$Month_{increment}$ (factor)

3.3.2 Model building and selection

A detailed description of the LMEM process used to analyse growth within and among regions is given in appendix section A.3.2. In brief, models describing somatic growth (increment width) within and among regions were fitted according to Morrongiello and Thresher (2015) whereby, intrinsic, random and extrinsic variables (see Table 3.1) were fitted in sequence. At each stage of the process, competing intrinsic, random and extrinsic models were ranked and the optimal model describing growth was identified through model selection criteria (Burnham and Anderson 2002). In this case, Akaike's information criterion corrected for small sample sizes (AIC_c) rescaled as the difference between the lowest AIC_c and each other model (ΔAIC_c) was used. Substantial support for a model was found if the ΔAIC_c was > 2 (Burnham and Anderson 2002).

Optimum models were refitted using restricted estimates of maximum likelihood (REML) to obtain unbiased parameter estimates (Zuur et al. 2009). Conditional and marginal R^2 were calculated as per Nakagawa and Schielzeth (2013) so that the proportion of variance explained by the fixed ($R^2_{lmm(marginal)}$) and random effects ($R^2_{lmm(conditional)}$) could be estimated

for each optimal model. The intra-class correlation coefficient (ICC) was calculated as a measure of correlation among growth increments from individuals (Fish_ID), inter-annually ($Month_{increment}$), among $Hatch_{months}$ and among cohorts ($Date_{migration}$). The ICC is effectively a measure of the strength of growth correlation and is further referred to as "growth synchrony" (Morrongiello and Thresher 2015).

Predicted effects of the most influential fixed effects predictors were plotted with 95% confidence intervals (CI) of the predicted values. Plots of the most important random effects were derived by extracting best linear unbiased predictors (BLUPs) from the appropriate model within and among regions. In instances where a random effect was non-significant, the results are shown in Appendix 3 .All statistical analyses were performed in R 3.2.2 (R Core Development Team 2015). LMEM were fit using the lme4 package (Bates et al. 2015). Model ranking and selection were done using AICcmodavg package.

3.4 Results

3.4.1 Otolith processing

In total, 569 fish were successfully aged with increment measurements obtained. A breakdown of the numbers of fish used in this analysis is shown in Table A.3.1. The daily increment pattern was usually clear and easily discerned (Fig. 3.1). However, individuals from Canterbury had a notably more complex microstructure with sub-daily rings and multiple check marks evident (Fig. A.3.3.).

The relationship between fish-length and otolith-width indicated linear and proportional relationships ($R^2 = 0.66$, $p < 0.001$) meaning overall, otolith size (width) is a good predictor of fish size (Fig. A.3.4). The strength of the relationship between fish-length and otolith-size found for inanga is appropriate for somatic growth studies (Morrongiello et al. 2014). Region-specific fish-length otolith-size relationships were not derived because there was relatively little variation in body size (see Chapter 2) to derive a regression for each region.

3.4.2 Developmental trajectory

Overall, increment widths ranged from 1.5 µm to 2.8 µm across regions. From plots of age-dependent growth rates, distinct growth phases were identifiable in most regions (Fig. 3.2). A slow growth phase was evident in the early to intermediate portion of life among South Island

regions. This demarcates the 'larval duration stage'. Growth generally increased rapidly in the intermediate to later stages followed by a plateau.

The length of these growth phases differed among regions. Growth was slow during the first 50 d of life in Golden Bay (Fig. 3.2). A period of rapid growth then occurred until a plateau at 120 d, just prior to inward migration (Fig. 3.2). The growth trajectory was similar in Buller although the larval duration stage was longer (approx. 80 d) and offset by approximately 30 d (Fig. 3.2). The slow-growth metamorphic phase took approximately 30 d in both regions. Age-dependent growth in Canterbury was consistently slower and relatively constant in comparison. No distinct increase prior to, or at inward migration was found for post-larvae in Canterbury (Fig. 3.2). The growth trajectory in the Bay of Plenty was more linear by comparison. No distinct slow growth phase was found but growth was slow up to 20 d (Fig. 3.2).

3.4.3 Sources of growth variation within regions

3.4.3.1 Among individuals

Sources of growth variation, as well as the magnitude of their contribution, differed among regions. Within each region, growth among individuals was highly variable (Table A.3.2). In each region, some individuals had higher or lower daily growth rates and were consistently faster or slower growing throughout their pelagic phase (Table 3.2). Individual differences in daily growth rates contributed more to growth variation than different growth:age slopes for each individual fish (Table 3.2). The magnitude of inter-individual variation differed among regions. Growth among individual fish was the most variable in the Bay of Plenty, but was over two times less variable in Buller (Table A.3.2).

The correlations found between Fish_ID random intercepts and growth:age slopes (Table 3.2) show that among-individual differences in daily somatic growth rates have a marked effect on larval growth trajectories. In the Bay of Plenty, Buller and Canterbury, fish with higher daily growth rates had shallower age-dependent growth slopes. On the other hand, individuals with lower daily growth rates had steeper age-dependent growth slopes. The magnitude of this effect was strongest in Buller (Corr = -0.47) and weakest in the Bay of Plenty (Corr = -0.17).

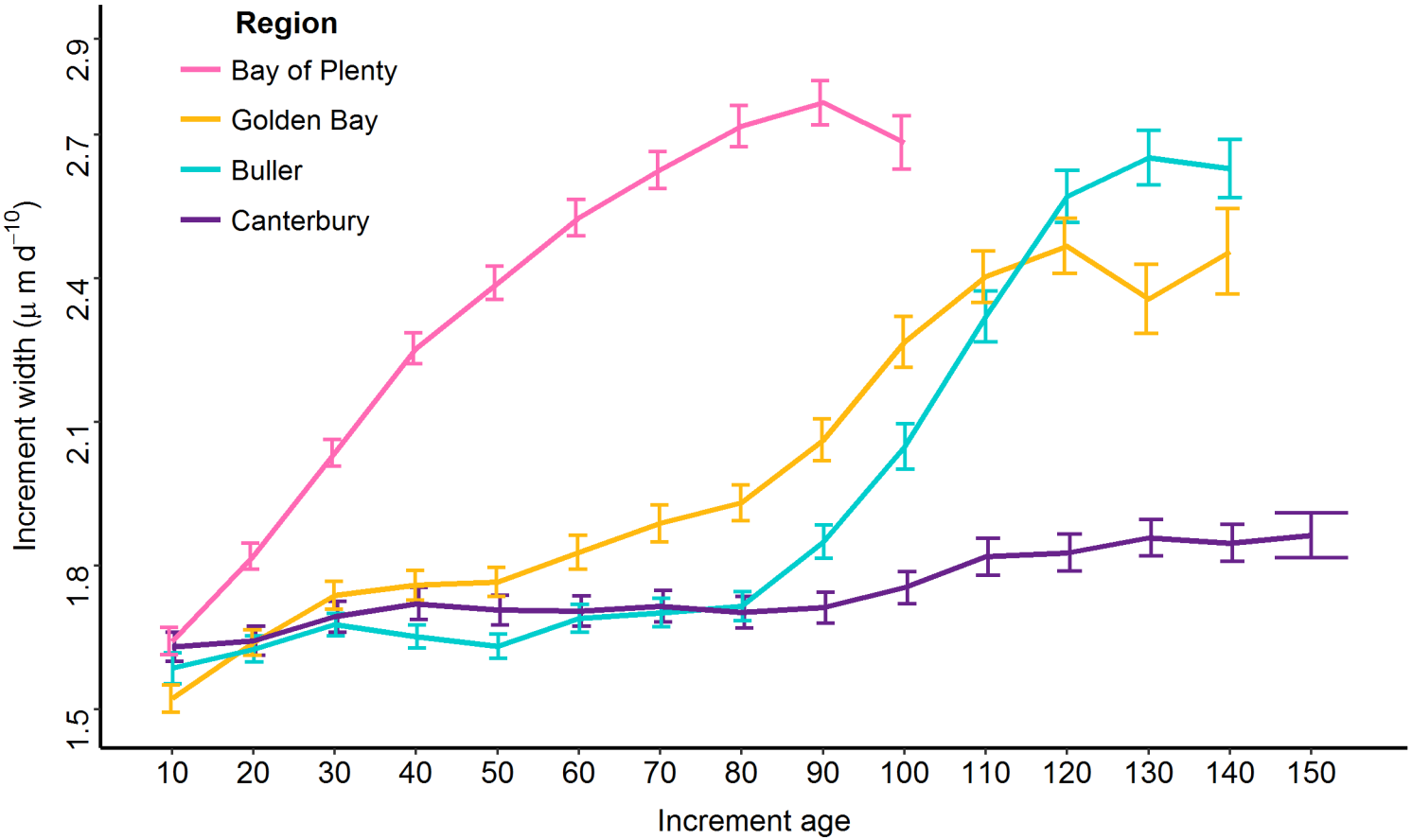


Figure 3.2. Mean age-dependent otolith increment width (± SE) among regions. Growth trajectories (µmd^{-10}) are plotted up to the average age of post-larvae at migration in each region.

In Golden Bay, the opposite pattern was found although the strength of this correlation was weak (Corr = 0.12). Here, individuals with higher than average daily growth rates had steeper age-dependent growth slopes, but those with lower than average daily growth rates had shallow age-dependent growth. The intra-class correlation (ICC) for within fish growth synchrony was relatively low and only 17%- 22% of within region growth variation was due to correlations within individuals (Table 3.2). Random intercepts and slopes for individual fish were included in all subsequent random effects models for each regions dataset.

3.4.3.2 Seasonal

$Month_{increment}$ (temporal environmental proxy) was the second largest source of growth variation in most regions (Table 3.2) and was included in each region's optimal random effects model (Table 3.3). Random effects plots show synchronous growth patterns across regions (Fig. 3.3). Growth was systematically lower during the winter months (June-August), but generally higher in autumn (March-May) and spring (September-November) (Fig. 3.3). Temporal growth synchrony was considerably greater in Buller (ICC= 0.27) compared to each other region (Table 3.2). Furthermore, inter-annual growth variation in Buller contributed considerably more to growth variation than any other region (Table 3.2).

Table 3.2. Variance components (± SD) from optimum random effects models identified for each region. 1 |y denotes random intercepts. x|y denotes random intercepts and slopes. $A_i = Age_{increment}$, C = Cohort, $H_m = Hatch_{month}$, $M_i = Month_{increment}$. Fixed effects fitted are $Age_{increment} + Age_{migration}$ for each random effects model. Corr = correlation statistic between intercepts and slopes. Intra-class correlations are reported for random intercept only models.

Random effects	**Bay of Plenty**	**Golden Bay**	**Buller**	**Canterbury**
1|Fish_ID	0.006 (0.083)	0.008 (0.089)	0.009 (0.094)	0.007 (0.084)
A_i|Fish_ID	0.001 (0.027) Corr = - 0.17	0.0003 (0.018) Corr = 0.15	0.001 (0.017) Corr = - 0.47	0.0002 (0.014) Corr = - 0.21
1|M_i	0.004 (0.065)	0.005 (0.070)	0.015 (0.12)	0.003 (0.055)
A_i|M_i	-	0.0001 (0.012) Corr= 0.80	-	-
1|H_m	-	0.003 (0.056)	-	-
1|C	0.001 (0.032)	-	-	0.003 (0.056)
A_i|C	0.001(0.017) Corr = 0.68	-	-	-
Residual	0.023 (0.153)	0.024 (0.154)	0.030 (0.17)	0.025 (0.159)
Intra-class correlation				
1|Fish_ID	0.220	0.225	0.173	0.198
1|H_m	-	0.137	-	-
1|M_i	0.080	0.093	0.274	0.091
1|C	0.065	-	-	0.083

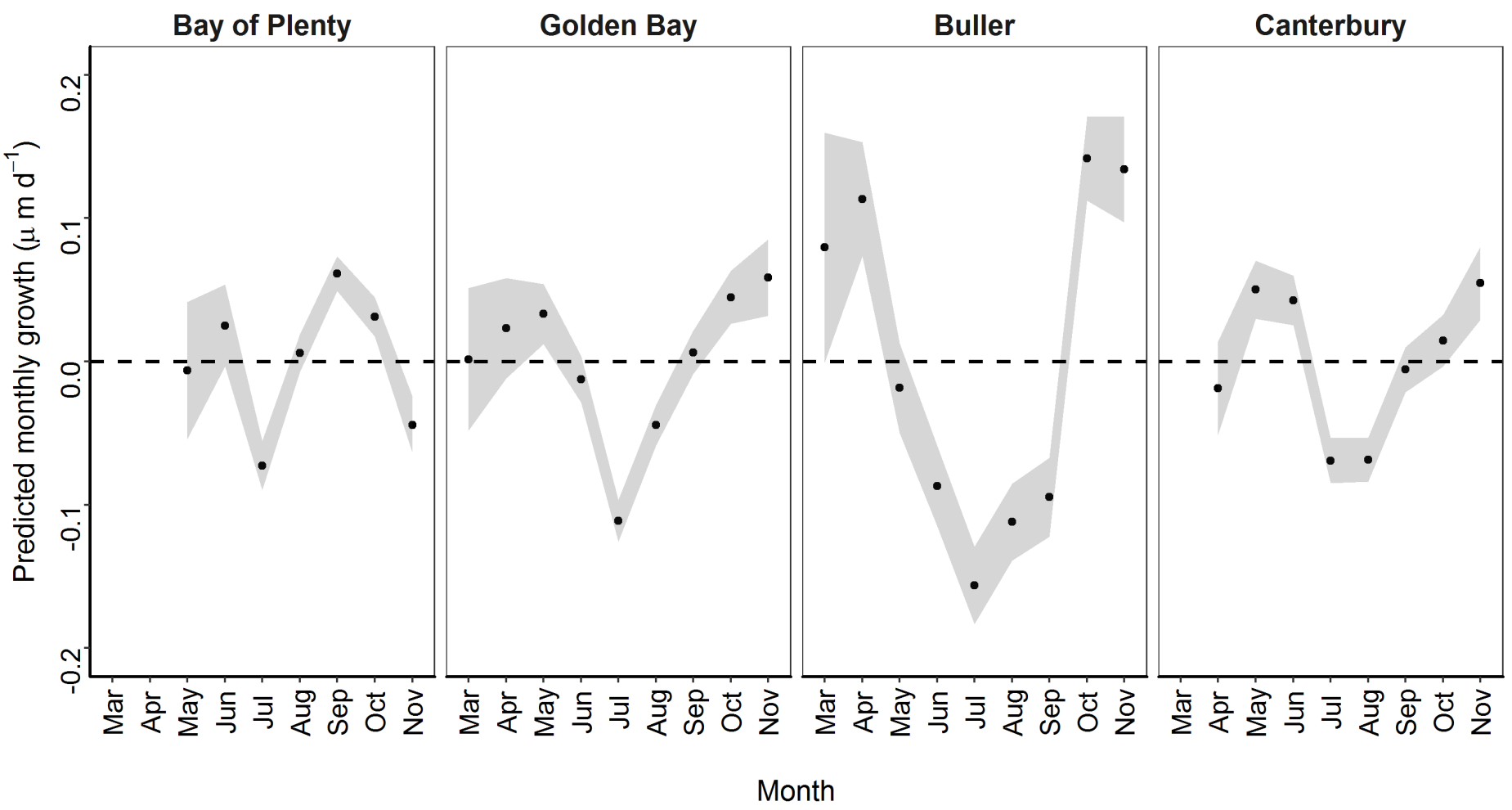


Figure 3.3. Random effects plot of average inter-annual growth variation within each region. The dashed line represents average growth (fixed effect model intercept) for each region. Bands are ± SE of the best linear unbiased predictors (BLUPs). Fixed effects fitted are $Age_{increment}$ + $Age_{migration}$ for each regions model.

Table 3.3. Model selection results for the optimal random effects structure within each region. Fixed effects fitted are $Age_{increment}$ + $Age_{migration}$ for each random effects model. 1|y denotes random intercepts. x|y denotes random intercept and slopes. A_i = $Age_{increment}$, C = Cohort, H_m = $Hatch_{month}$, M_i = $Month_{increment}$. LL = log likelihood. K = model complexity**.** ΔAIC_c is the difference between each model. The optimal random effects models are highlighted in **bold.**

Region	Model #	Random effect	K	ΔAIC_c	LL	AIC_c
Bay of Plenty	**B1**	**$1|M_i + 1|C$**	**9**	**0**	**530.31**	**-1042.52**
	B2	$1|M_i + 1|H_m + 1|C$	10	2.02	530.31	-1040.50
	B3	$1|M_i + 1|H_m$	9	5.63	527.50	-1036.90
	B4	$1|M_i$	8	8.62	524.99	-1033.90
	B5	1|C	8	55.85	501.37	-986.67
	B6	$1|H_m + 1|C$	9	57.87	501.37	-984.65
	B7	$1|H_m$	8	60.47	499.06	-982.05
	B8	Ai|Fish_ID	7	67.15	494.72	-975.37
Golden Bay	G1	$1|M_i +1|H_m + 1|C$	10	0	520.83	-1021.53
	G2	**$1|M_i + 1|H_m$**	**9**	**1.00**	**519.32**	**-1020.53**
	G3	$1|M_i + 1|C$	9	1.77	518.93	-1019.76
	G4	$1|M_i$	8	16.22	510.69	-1005.30
	G5	$1|H_m + 1|C$	9	98.55	470.54	-922.98
	G6	1|C	8	99.79	468.91	-921.74
	G7	$1|H_m$	8	99.94	468.84	-921.59
	G8	A_i|Fish_ID	7	111.38	462.11	-910.15
Buller	**Bl1**	**$1|M_i$**	**8**	**0**	**460.70**	**-905.32**
	Bl2	$1|M_i + 1|H_m$	9	1.72	460.84	-903.60
	Bl3	$1|M_i + 1|C$	9	1.96	460.72	-903.36
	Bl4	$1|M_i + 1|H_m + 1|C$	10	3.73	460.85	-901.59
	Bl5	1|C	8	278.43	321.48	-626.89
	Bl6	$1|H_m + 1|C$	9	280.45	321.48	-624.87
	Bl7	$1|H_m$	8	283.63	318.88	-621.69
	Bl8	A_i|Fish_ID	7	288.92	315.23	-616.40
Canterbury	**C1**	**$1|M_i +1|C$**	**9**	**0**	**562.17**	**-1106.24**
	C2	$1|M_i + 1|H_m +1|C$	10	2.02	562.17	-1104.22
	C3	$1|M_i + 1|H_m$	9	4.65	559.84	-1101.59
	C4	$1|M_i$	8	9.50	556.41	-1096.74
	C5	1|C	8	108.04	507.14	-998.20
	C6	$1|H_m + 1|C$	9	110.06	507.14	-996.18
	C7	$1|H_m$	8	112.97	504.67	-993.27
	C8	A_i|Fish_ID	7	117.61	501.35	-988.64

3.4.3.3 Cohorts

Cohort-specific differences in mean daily growth (intercept) contributed less to growth variation in Golden Bay and Buller, but was a significant source in the Bay of Plenty and Canterbury (Table 3.3). In the Bay of Plenty and Canterbury, fish that migrated inward during early- and late-September had the lowest growth, and fish that migrated in late-October had the highest growth (Fig. 3.4). Growth synchrony (ICC) was low among cohorts in both regions (Table 3.2).

In the Bay of Plenty, there was considerable evidence for variable age-dependent growth trajectories among cohorts (Table A.3.3). The strong positive correlation between cohort random intercepts and age slopes (Corr = 0.68, Table 3.2) indicates that cohorts of fish with higher daily growth rates had steeper age-dependent growth trajectories.

In Golden Bay and Buller, there was relatively equal support for growth variation among hatch-months as there was among cohorts (Table 3.3). The most complex models did not show a significant improvement in the ΔAIC_c in either region (Model G1 and Model Bl14). Therefore, although there was some evidence that growth varied among cohorts, there was slightly more evidence that it varied among hatch-months in these regions (Table 3.3; Fig. A.3.5).

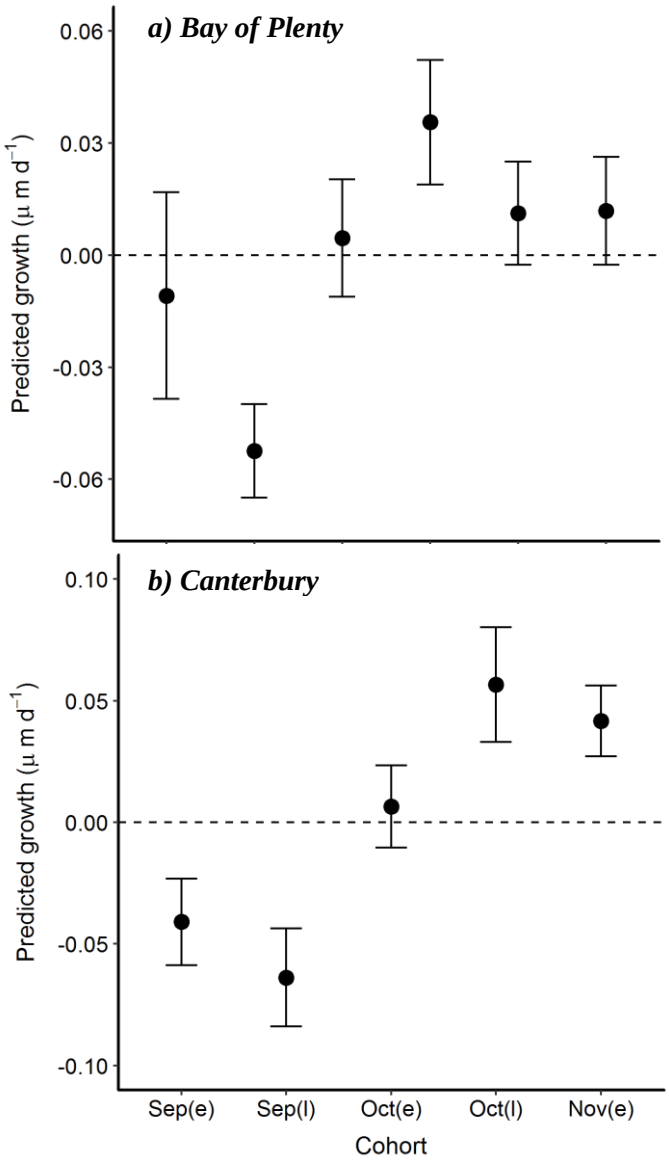


Figure 3.4. Random intercept plots of average daily growth variation (μmd^{-1}) among cohorts within **a)** Bay of Plenty and **b)** Canterbury. In each panel the dashed line represents average growth (fixed effect model intercept) for each regions model. Bands are ± SE of the best linear unbiased predictors (BLUPs). Fixed effects fitted are $Age_{increment} + Age_{migration}$ for each regions model. Note different scales on y-axes.

3.4.3.4 Hatch-months

Overall, differences among hatch-months were the least significant source of variation within each region. Hatch-month did not feature in the optimal model for Bay of Plenty, Buller or Canterbury (Table 3.3). Daily growth rates were therefore relatively consistent among hatch-months in each of these regions (Fig. A.3.6).

In Golden Bay, variation among hatch-months was 16 times more likely to explain growth variation than the simpler model with terms accounting for variation due to inter-annual effects and individual fish (Model G2; Table 3.3). Fish that hatched in March-May had significantly lower mean daily growth compared to those hatched in June-August (Fig. 3.5). There was strong evidence that age-dependent growth trajectories did not vary among hatch-months (Table A.3.3). The ICC showed low levels of growth synchrony within hatch-months in Golden Bay (Table 3.2).

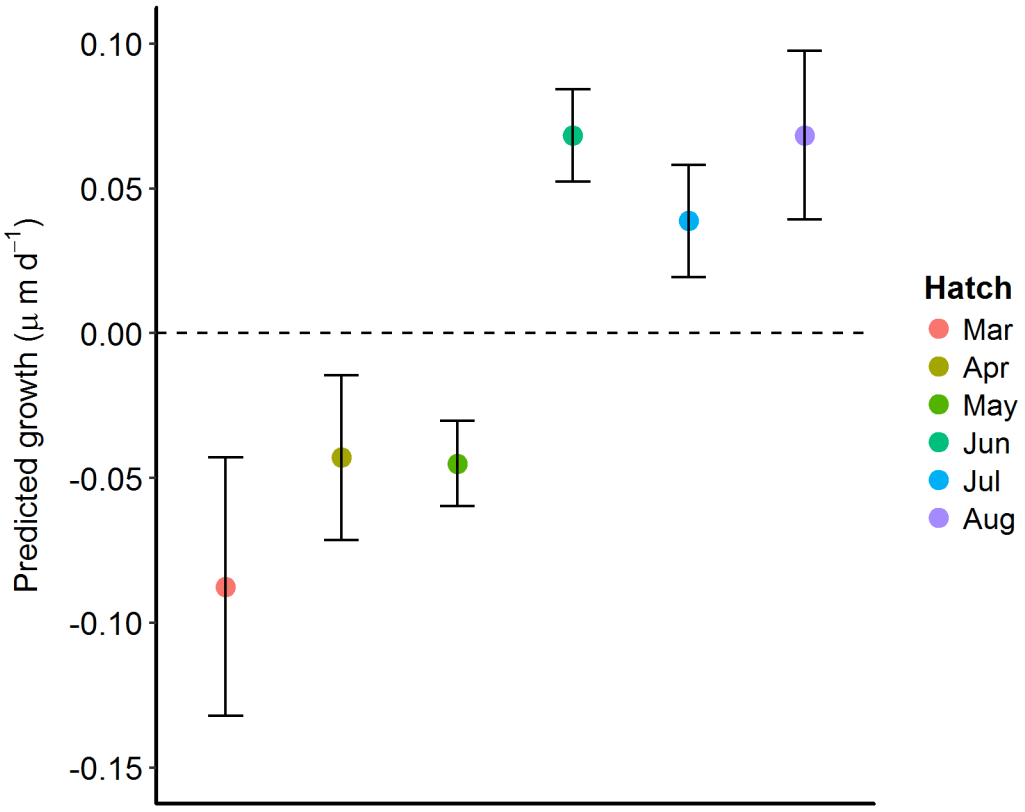


Figure 3.5. Random intercept plots of average daily growth variation (μmd^{-1}) among hatch-months within Golden Bay. In each panel the dashed line represents average growth (fixed effect model intercept) for each regions model. Bands are ± SE of the best linear unbiased predictors (BLUPs). Fixed effects fitted are $Age_{increment}$ + $Age_{migration}$.

3.4.4 Intrinsic effects

3.4.4.1 Ontogeny

The most important factor determining growth rate variation for inanga within each region was ontogeny (i.e., growth rates increased with age throughout pelagic development). Daily growth and the age-dependent growth trajectories varied according to age at inward migration (shown through the inclusion of the $Age_{increment}$*$Age_{migration}$ interaction term; Table A.3.4). The importance and magnitude of these ontogenetic components for growth varied among regions. In Canterbury, there was weak evidence that age-dependent growth rates differed among ages ($\Delta AIC_c < 2$; Table A.3.4). Instead, growth increased with ontogeny (Table 3.4) and older larvae had lower daily growth rates (Table 3.4; Fig. 3.6).

In Buller, variation in age-dependent growth among age at inward migration was 8 times more likely to explain growth than the simpler model with an additive term for $Age_{migration}$ (Table A.3.4). Similar results were found in the Bay of Plenty and Golden Bay, but the magnitude of this effect was weaker ($\Delta AIC_c < 4$; Table A.3.4).

Overall, younger post-larvae at inward migration had higher age-dependent growth compared to those that were older at migration in Bay of Plenty, Golden Bay and Buller (Fig. 3.6). Significant differences between younger and older post-larvae became apparent after approximately 40 d development in Bay of Plenty and Golden Bay (Fig. 3.6). In Buller, differences among older and younger post-larvae became apparent at approximately 70 d (Fig. 3.6). In each region, the growth trajectories of younger and older post-larvae diverged within the range of 1.8 – 2.0 μmd^{-10} (as shown by the 95% CI; Fig. 3.6). Significantly steeper age-dependent growth rates of younger post-larvae were maintained through to inward migration in each region (Fig. 3.6).

The magnitude of increase in age-dependent growth ($Age_{increment}$) differed among regions. Post-larvae in the Bay of Plenty were the fastest growing ($\beta = 0.058$, SE = 0.008, t = 7.098) while those in Canterbury, the slowest ($\beta = 0.012$, SE = 0.003, t = 4.63; Table 3.4). Age-dependent growth rates in Golden Bay ($\beta = 0.037$, SE = 0.003, t = 12.47) and Buller ($\beta = 0.039$. SE = 0.003, t = 13.93) regions increased at an intermediate rate relative to the Bay of Plenty and Canterbury and largely overlapped each other (Table 3.4). Older post-larvae ($Age_{migration}$) in Buller showed the steepest declines in growth and Canterbury the shallowest (Table 3.4). The magnitude of declining growth in older larvae was comparable between Bay of Plenty and Golden Bay (Table 3.4).

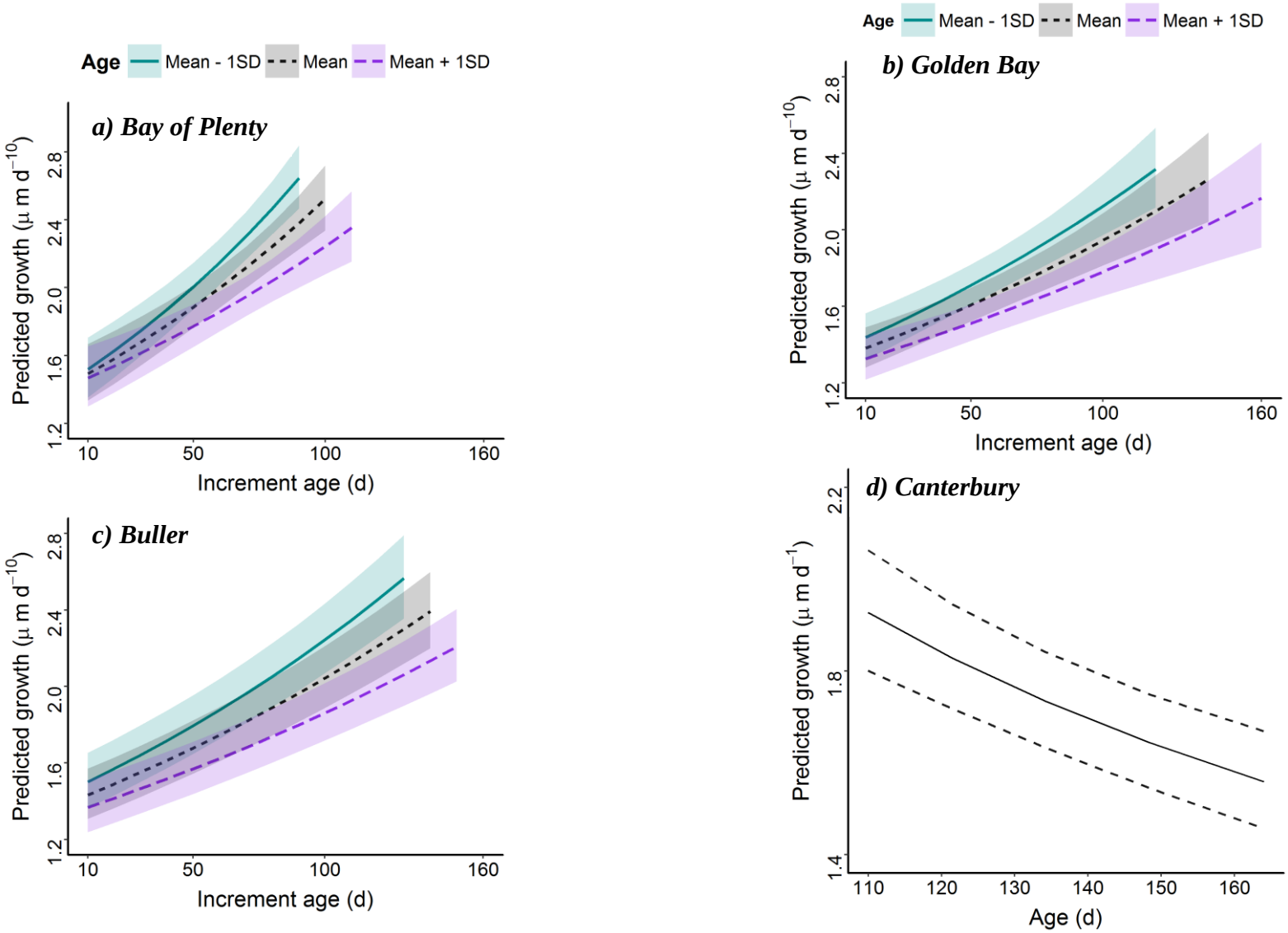


Figure 3.6. Intrinsic fixed effects results from optimal models for each region. Panels **a), b)** and **c)** show the interaction $Age_{increment}$ *$Age_{migration}$ for Bay of Plenty, Golden Bay and Buller. In these panels, growth is plotted for the average age of post-larvae at migration ± SD to visualise the interaction term. Panel **d)** shows the $Age_{migration}$ fixed effect estimates for Canterbury. In each panel **(a-d),** lines are the fitted values from the optimal intrinsic fixed effects model. Bands are 95% CI of the fitted values.

Table 3.4. Fixed effect parameter estimates (± SE) and test statistics for the optimal model describing growth variation within each region. All models are fitted with the optimal fixed and random effects structures identified for each region.

Intrinsic fixed effects		Estimate	t-value
Bay of Plenty	**Intercept**	0.808 (0.030)	26.335
	$Age_{increment}$	0.058 (0.008)	7.098
	$Age_{migration}$	-0.678 (0.062)	-10.933
	$Age_{increment}$:$Age_{migration}$	-0.079 (0.018)	-4.358
Golden Bay	**Intercept**	0.661 (0.029)	22.95
	$Age_{increment}$	0.037 (0.003)	12.47
	$Age_{migration}$	-0.762 (0.094)	-8.14
	$Age_{increment}$:$Age_{migration}$	-0.034 (0.017)	-2.00
Buller	**Intercept**	0.695 (0.040)	17.24
	$Age_{increment}$	0.039 (0.003)	13.93
	$Age_{migration}$	-0.910 (0.084)	-10.77
	$Age_{increment}$:$Age_{migration}$	-0.052 (0.015)	-3.35
Canterbury	**Intercept**	0.550 (0.029)	19.05
	$Age_{increment}$	0.012 (0.003)	4.63
	$Age_{migration}$	-0.534 (0.086)	-6.22

3.4.5 Extrinsic effects

3.4.5.1 Rivers

There was weak evidence that ontogenetic growth patterns varied among rivers in each region. In the Bay of Plenty, the highest-ranking model indicated that the $Age_{increment}$*$Age_{migration}$ interaction varied among rivers but ΔAIC_c was low (Table A.3.6). Instead, there was significant evidence that mean growth rates varied among rivers in the Bay of Plenty with post-larvae that migrated into the Kaituna River having higher mean daily growth compared to the Wairoa River but not the Waimapu River (β = -0.035, SE = 0.019, t = -1.832; Table 3.5).

In Canterbury, the evidence for differences between rivers was stronger as the ΔAIC_c was > 9 (Table A.3.5). Results showed that older fish at inward migration had lower daily growth rates in the Ashley compared to the Waimakariri River (Table 3.5). In Golden Bay and Buller, there was less evidence that the intrinsic ontogenetic components ($Age_{increment}$ and $Age_{migration}$) varied among rivers (Table A.3.5).

Table 3.5. Fixed effect parameter estimates (± SE) and test statistics for optimal extrinsic model describing growth variation within each region. All models are fitted with the optimal intrinsic fixed and random effects structures identified previously.

Extrinsic fixed effects		Estimate	t-value
Bay of Plenty	**Intercept** (Wairoa River)	0.784 (0.033)	23.710
	$Age_{increment}$	0.058 (0.008)	7.044
	$Age_{migration}$	-0.719 (0.064)	-11.213
	Waimapu River	0.013 (0.019)	0.717
	Kaituna River	0.049 (0.018)	2.740
	$Age_{increment}$: $Age_{migration}$	-0.080 (0.018)	-4.423
Canterbury	**Intercept** (Waimakariri River)	0.522 (0.032)	16.390
	$Age_{increment}$	0.013 (0.003)	4.650
	$Age_{migration}$	-0.373 (0.104)	-3.570
	Ashley River	0.047 (0.017)	2.700
	$Age_{migration}$:Ashley River	-0.367 (0.135)	-2.500

Overall, a considerable amount of variation in growth was explained by the optimal models for each region. Most growth variation was due to intrinsic ontogenetic effects (Table 3.6). The inclusion of increasingly complex random effects structures captured at least an additional 20% more variation than intrinsic ontogenetic sources alone. The proportion of variation explained by both fixed intrinsic and extrinsic effects as well as random effects in Canterbury was the lowest (Table 3.6).

Table 3.6. The proportion of variation (R^2) explained by the optimal intrinsic fixed (marginal) and fixed plus random effects (conditional) model within each region are shown. The proportion of variation explained by the addition of extrinsic effects to the optimal fixed effects on the marginal and conditional R^2 is shown.

Region		Model #	Marginal	Conditional
Bay of Plenty	Intrinsic	I1	0.46	0.65
	Extrinsic	E1	0.47	0.65
Golden Bay	Intrinsic	I4	0.37	0.62
	Extrinsic	E5	-	-
Buller	Intrinsic	I7	0.39	0.63
	Extrinsic	E9	-	-
Canterbury	Intrinsic	I10	0.14	0.42
	Extrinsic	E13	0.17	0.43

3.4.6 Among regions

Age-dependent growth trajectories within hatch-months and cohorts among regions are shown in Fig. 3.7. For most hatch months and cohorts, inanga in the Bay of Plenty had higher age-dependent growth rates compared to the South Island regions.

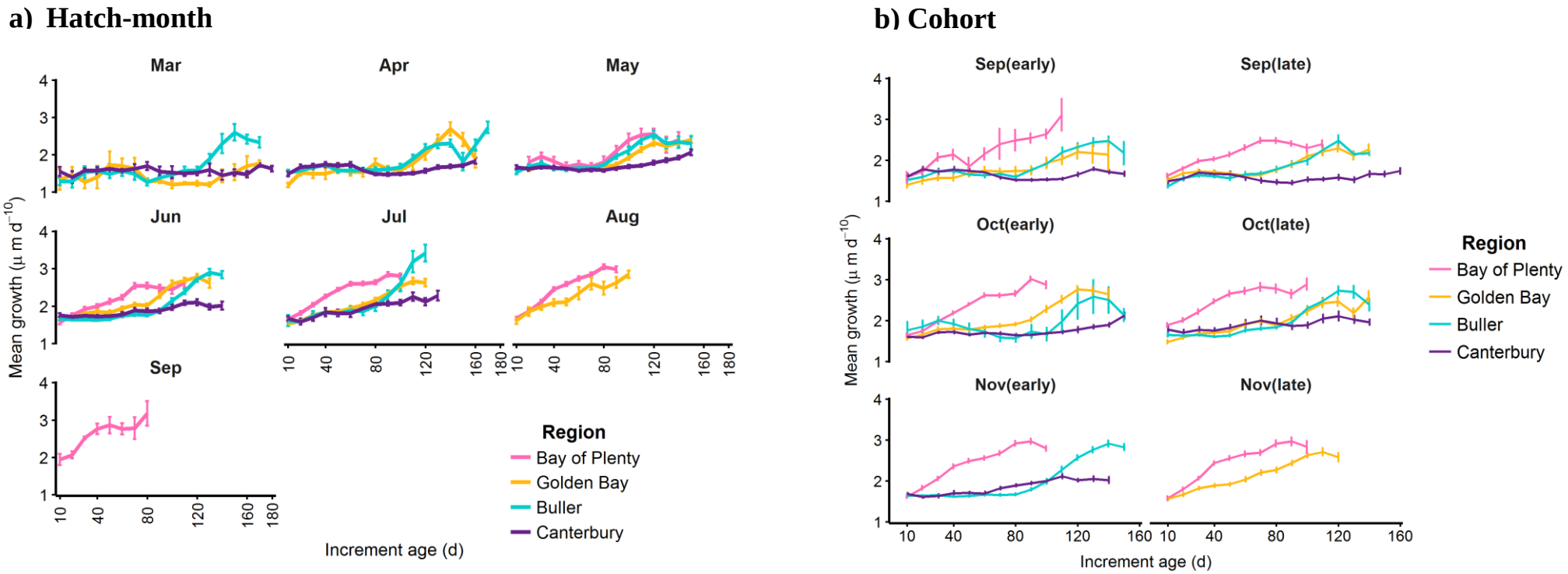


Figure 3.7. Mean (µmd^{-10}) age-dependent otolith increment widths (±SE) within **a)** hatch-months and **b)** cohorts among regions. Growth trajectories are plotted up to the average age of post-larvae at migration in each hatch-month and cohort (early and later) for each region.

In total, 26 models of increasing random effects complexity (intercepts, nesting and slopes) were built to understand among-region growth variation (Table A.3.6, Table A.3.7). Similar to the within region models, inter-individual variation was the most significant source of growth variation among regions (Table 3.7).

3.4.6.1 Seasonal

Among regions, daily growth rates varied substantially in any given month (temporal environmental proxy), as well as growth:age slopes (Table A.3.8). This shows that inanga are not necessarily displaying a uniform growth signal among regions in any given month of the year. Although there was considerable overlap among regions, inanga had significantly lower daily growth rates (Fig. 3.8) and were slower growing (shallower age-dependent growth slopes) from September to November in Canterbury compared to fish in the Bay of Plenty. Temporal growth variation between Golden Bay and Buller was similar although Golden Bay had lower growth in March and April by comparison.

3.4.6.2 Hatch-months

Fish hatched in the same month grew at different rates across regions. June-hatched fish collected in the Bay of Plenty had lower daily growth rates than June-hatched fish in each other region (Fig. 3.8). July-hatched fish were slower growing in Canterbury compared to those in each other region (Fig. 3.8). However, by and large, a strong directional trend in growth was not evident and there was a large amount of variation around the predicted values (Fig. 3.8).

3.4.6.3 Cohorts

Across regions, growth did not vary substantially among cohorts. Instead, there was a region-wide growth signal (Table 3.7). Overall, fish that migrated inward during early- and late-September had significantly lower growth compared to those in October and November (Fig. A.3.7).

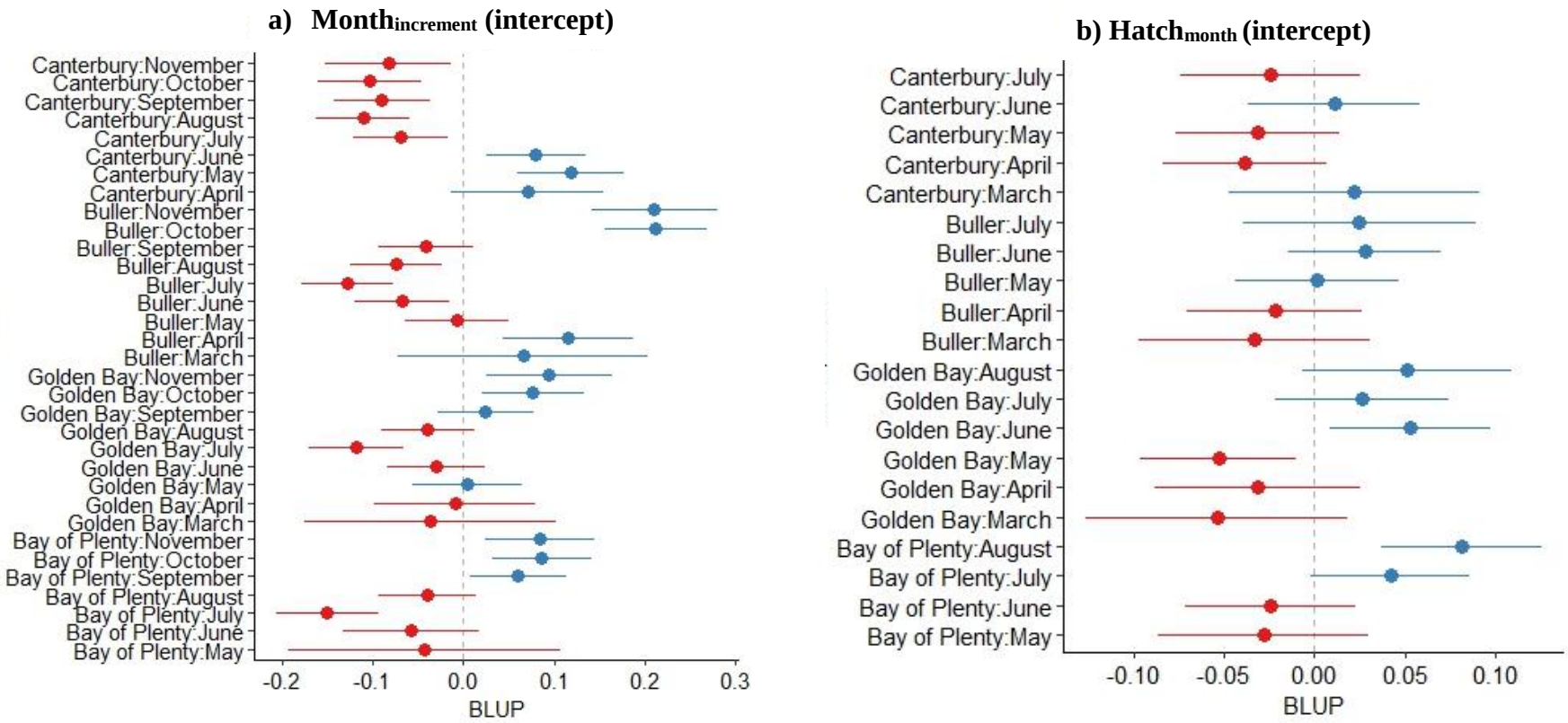


Figure 3.8. Random effects plot of average **a)** inter-annual ($Month_{increment}$) and **b)** $Hatch_{month}$ growth variation across regions. Red represents lower- and blue higher-predicted values. Dashed line represents average growth (fixed effect model intercept) across regions. Bands are ± SE of the best linear unbiased predictors (BLUPs). Fixed effects fitted are $Age_{increment} + Age_{migration}$.

Table 3.7. Variance components (± SD) from optimum random effects model across regions. 1|y denotes random intercepts. x|y denotes random intercept and slopes. : denotes nested random effects A_i = $Age_{increment,}$ C = Cohort, H_m = $Hatch_{month}$, M_i = $Month_{increment,}$ R_g = Region.

Random effects	**Variance estimates**
1|Fish_ID	0.007 (0.086)
A_i|Fish_ID	0.003 (0.018) Corr = -0.09
1|R_g:Mi	0.004 (0.062)
A_i|R_g:Mi	0.004 (0.021) Corr = 0.39
1|R_g:H_m	0.002 (0.041)
A_i|R_g:H_m	0.0001 (0.011) Corr = 0.94
1|C	0.0005 (0.023)
1|R_g	0.002 (0.050)
Residual	0.0249 (0.157)

3.5 Discussion

Growth during the pelagic phase is a dynamic and complex part of the life cycle of inanga. In this study, otolith-derived somatic growth reconstructions coupled with linear mixed effects models were used to understand intrinsic and extrinsic sources of growth variation and how this varied within and among regions in New Zealand. Results showed that, intrinsic ontogenetic effects were the most important component of growth. The dominant growth pattern was an allometric increase in age-dependent growth that varied among regions. Distinct life-stage transitions (larval, competency and metamorphosis) were found from age-related changes in somatic growth in most regions.

Within most regions, significant growth variation was found among individuals and cohorts, but to a lesser extent among hatch-months. Older post-larvae at inward migration had lower daily growth rates in all regions. Additionally, age-dependent growth trajectories varied among age at inward migration in most regions. Younger post-larvae completed their larval stage duration quicker because they were faster growing during the early phase of life compared to older post-larvae at inward migration. Furthermore, age-dependent growth trajectories of younger and older post-larvae diverged at a relatively consistent growth rate (1.8-2.0 μmd^{-10}) across regions. However, there were region-specific differences when these growth trajectories diverged: earlier for Bay of Plenty (approx. 50 d old) and later for Golden Bay and Buller (approx. 70 d old). Within regions, growth showed a seasonal cycle and was consistently lowest during winter. This trend is consistent with seasonal variation in SST and productivity that occurs from autumn through to spring across the country.

Post-larval growth was considerably more complex and variable among regions than within regions. This suggests that inanga are more responsive to processes occurring within regions than among regions. Among regions, significant sources of growth variation were among individuals, hatch-months and seasons, but not among cohorts. Growth followed a distinct geographic trend associated with latitude. Post-larvae were faster growing in the Bay of Plenty relative to Canterbury. The growth trajectories of post-larvae in Golden Bay and Buller largely overlapped, but were intermediate relative to the Bay of Plenty and Canterbury. Inter-annual growth variation further showed that in any given month of the year, growth was considerably higher in the Bay of Plenty relative to Canterbury. I hypothesise that SST and productivity gradients along with regional variation in oceanography are partly driving

different growth patterns across regions and are an important component of the population dynamics of inanga.

3.5.1 Intrinsic drivers of growth

Previous studies have assumed that growth during the pelagic development of inanga occurs at a constant daily rate (McDowall et al. 1994, Rowe and Kelly 2009, Barbee et al. 2011). Instead, results showed distinct growth phases. These growth phases (larval, competency and metamorphosis) align with developmental transitions commonly found among other species with pelagic early life stages such as sardine (*Sardina pilchardus* (Schismenou et al. 2016) or tarakihi (*Nemadactylus macropterus*) (Bruce et al. 2001). Studies to date refer to the total time spent by inanga in the pelagic environment as their "larval dispersal" period (Hale et al. 2009, Rowe and Kelly 2009, Barbee et al. 2011, Hickford and Schiel 2016). Based on my results, larval development and the associated dispersive stages are likely much shorter than assumed previously. Faster growing fish completed their larval stages quicker, progressed to the competent stages earlier, and maintained higher growth rates until inward migration at a younger age relative to slower growing older fish. This shows that processes influencing early growth are important determinants for 1) the length of time spent in their developmental stages and 2) age at inward migration to freshwaters.

Understanding the developmental trajectory of inanga has important consequences for their population dynamics because it is during the larval stage that the propensity to disperse is greatest (Hickford and Schiel 2016). Following hatching, amphidromous larvae are considered largely passive because of their small size and poorly developed sensory abilities (McDowall 2009, Jarvis and Closs 2015). As such, their initial dispersal kernel is dictated by hydrology and other abiotic conditions. Nothing is known about their behaviours once they transition to a more competent life-stage in the pelagic environment. However, it is widely shown that the dispersal abilities of fish are influenced, if not controlled, by ontogenetic changes in their behaviour, abilities and requirements (Leis 2007). Once competent, individuals can orientate themselves into freshwater plumes (Grimes and Kingsford 1996) or maintain a relatively stationary position by exhibiting diel vertical migrations (Fiksen et al. 2007). Such behaviours, and many more associated with development, can collectively restrict the spatial and temporal extent of dispersal of inanga.

There is an ever-growing body of research showing that amphidromous species disperse for a brief period in their larval life then make an ontogenetic migration back to freshwaters.

For example, otolith microchemistry studies show that most pelagic development occurs in inshore environments or freshwater plumes and not in the marine environment (Sorensen and Hobson 2005, Hogan et al. 2014, Shiao et al. 2015, Warburton 2015, Hogan et al. 2017). However, few studies have linked growth with the timing of these habitat transitions. A recent study by Hogan et al. (2017) showed that fast growth rates of *Awaous stamineus* during early life coincided with larval development in nearshore low salinity environments and not the marine environment. The transition in the growth trajectory of inanga following larval growth might coincide with ontogenetic movements of faster growing fish into alternative habitats (e.g., coastal waters or freshwater plumes). Ontogenetic migrations into more productive environments are likely advantageous because they facilitate increased energetic, metabolic and trophic demands (McDowall 2010) of faster growing fish. Furthermore, such ontogenetic migrations also ensure individuals are near the appropriate cues needed to migrate while giving them an opportunity to begin to adapt to less saline environments. In anadromous salmonids, pre-migratory stages move from the upper to the lower catchment or into estuaries in preparation for their seaward migrations. They do this because there is a "physiological and ecological window" for their migrations (McCormick et al. 1998). These windows are likely relevant for the migration of inanga.

Despite regional variation in age at inward migration, growth among younger and older fish consistently diverged at 1.8 – 2.0 μmd^{-10} in each region. This shows that there is a likely a growth rate threshold for migration. Among amphidromous species, weak correlations between size and age were hypothesised to be a result of 1) inter-individual growth variation, 2) a slow growth phase during dispersal and/or 3) a threshold size to migrate (Hoareau et al. 2007). However, no studies have explicitly identified a threshold growth rate for migration. Among diadromous species conditional or 'threshold traits' (Roff 1996) such as size, age or growth rate are widely known to underlie their migrations. For example, among migratory brown trout (*Salmo trutta*), faster growing individuals during their first year of life in the river migrated to the sea at a younger age compared to fish that were slower growing in early life (Marco-Rius et al. 2013). Growth rate thresholds are also related to their propensity to migrate to sea or remain resident in freshwater (Theriault and Dodson 2003) as well as the number of reproductive events (Halttunen et al. 2013). Growth thresholds for the inward migration of inanga are a critical part of their early life history dynamics. These thresholds are likely a function of a suite of complex, yet poorly understood interactions between intrinsic (e.g. metabolic rate, maternal effects, previous growth history) and environmental effects as well as a genetic component.

3.5.2 Linking reproduction with early life histories

For species with dispersive larvae, adult reproductive patterns are one of the most important components that can alter larval dispersal pathways and inter-population exchange (Treml et al. 2012). In this study, there was substantial growth variation among hatch-months across regions, particularly for winter-hatched fish. This result was largely driven by differences between the Bay of Plenty and each other region, but differences among South Island regions were also evident. One mechanism to explain this result is that the larval stages are structured by spatial and temporal variation in spawning times coupled with oceanographic processes (Fortier and Leggett 1983). Inanga spawn almost year round, from late-summer in the south through to early-spring in the north (Taylor 2002, Hicks et al. 2013). Within regions, spawning can also extend over a five month window (Stevens et al. 2016) and so there is some overlap among regions in spawning and thereby hatching times. Although the origins of larvae are unknown, the clear regional growth differences found, even for larvae hatched in the same month, show that larvae hatched at similar times are spending most of their pelagic-life in distinct water masses.

Interestingly, despite the temporal extent of hatching (over 5 months), growth was relatively consistent among hatch-months within most regions. Many studies show variable growth patterns among hatching dates driven by temporal variation in temperature, food, weather conditions etc. (Alemany et al. 2006, Baumann et al. 2006, Weber et al. 2015). Lejeune et al. (2016) show that for the amphidromous *Sicydium punctatum*, later-hatched fish had higher initial growth and spent less time in the pelagic environment than those hatched earlier. For inanga, the relationship between hatching and growth is complex and confounded by age at inward migration. Progressively later-hatched fish have higher age-dependent growth (i.e., they are faster growing, Fig. A.3.6). But, in each region, progressively later-hatched fish are on average younger at inward migration and so less time is spent growing in the pelagic environment. Although later-hatched fish have higher age-dependent growth rates, because they leave the pelagic earlier, their pelagic growth is comparable to earlier-hatched, slower growing fish that spend longer in the pelagic. Hogan et al. (2014) found similar results among amphidromous and non-migratory *Awaous stamineus*, but did not relate this to hatch date. They showed that, mean daily growth was consistent among migratory and non-migratory individuals, but those that developed in the marine environment were significantly faster growing and younger at migration.

3.5.3 Extrinsic factors driving growth

3.5.3.1 Inter-annual variation

Within regions, temporal variation in the abiotic environment was the most important extrinsic driver of growth variation. Temporally resolved "environmental proxies" showed that growth followed a cyclical pattern of higher and lower growth from autumn through to spring in each region. This cyclical pattern coincides with seasonal variability in sea surface temperatures (SST) and productivity found throughout the country (Hadfield and Sharples 1996, Murphy et al. 2001, Chang et al. 2003) but also corresponds with seasonal changes in photoperiod. Temporal growth variation was considerably weaker in the Bay of Plenty compared to Buller and of almost equal importance in Golden Bay compared to Canterbury. On the West Coast of New Zealand, strong seasonal fluctuations in primary productivity are driven by inputs from freshwater sources associated with precipitation (Schiel 2004) as well as upwelling and downwelling systems (Menge et al. 2003). Because the magnitude of seasonal variation in SST is generally weaker here (Fig. 1.2) productivity is probably the primary extrinsic factor driving seasonal growth variation in Buller. Likewise, seasonal trends found in Golden Bay are probably driven more so by productivity than SST because mean inter-annual SST is similar to Buller (Fig. 1.2). In Canterbury, the abiotic drivers were less evident because low SST and low productivity characterises this region. The weaker effect of seasonality on growth in the Bay of Plenty is typical of lower latitudes.

Among regions, temporal variation was also the most significant source of growth variation. The most distinct temporal differences in growth were between the Bay of Plenty and Canterbury. In most months of the year, SSTs are at least 5 ºC warmer in the Bay of Plenty relative to Canterbury (Fig. 1.2) and so regional growth variation is likely driven by temperature. Geographic and inter-annual variation in SST throughout New Zealand influences growth in other fish species (Gillanders et al. 2012, Trip et al. 2014). However, for larval and juvenile snapper (*Chrysophrys auratus*) in northern New Zealand, their growth rates were only weakly related to SST and so variation in food availability, habitat quality and predation pressure are likely driving growth variation (Sim-Smith et al. 2012). Temperature can affect larval growth directly via its effects on food consumption, growth efficiency, metabolic rate and oxygen uptake (Houde 1989). Indirect effects include the length of the growing season which varies with latitude (proxy for temperature) meaning there is a longer window for growth at low latitudes (Conover 1992). Other indirect effects that co-vary with temperature include food quality and quantity, abundance of predators, density-dependence and habitat quality

which can all impact on growth depending on how an individual interacts with these factors (Richardson 2008, Allan et al. 2015).

One of the primary advantages of using mixed models for otolith increment width studies is that, during the modelling process, intrinsic ontogenetic effects were accounted for prior to examining temporal (or any other) sources of growth variation. Among amphidromous species, attempts to relate pelagic growth to abiotic conditions are few. In Teichert et al.'s (2016) study on the relationship between age-dependent growth and SST of an amphidromous gobiid, they failed to account for ontogenetic effects inherent in growth. As a result, the effects of temperature on growth are not immediately apparent. Relating pelagic growth histories of amphidromous species with environmental variables is becoming increasingly important especially in light of climate change (Murphy and Cowan 2007, Teichert et al. 2016) and studies that wish to understand what drives plasticity in their migratory behaviours (Augspurger et al. 2016). Future studies seeking to couple growth with the environment must take the appropriate measures to partition growth accordingly.

3.5.3.2 Cohorts

Within regions, there was significant growth variation among cohorts in the Bay of Plenty and Canterbury. Results showed that post-larvae with the highest growth rates migrated in late-October while slower growing fish migrated in September in these regions. Barbee et al. (2011) found a similar result among Australian populations of inanga whereby inward migrating post-larvae were significantly slower growing in September compared to those in January. One hypothesis to explain this result is that growth is not just a function of hatching time but is also likely driven by temporal variation in abiotic conditions at a more local scale. For amphidromous species, precipitation and river flows (McDowall 1995), along with seasonal changes in temperature (Shiao et al. 2015) are important environmental cues triggering their inward migration. These cues, particularly flows, are sometimes unpredictable and can vary seasonally and regionally in New Zealand (Jowett and Duncan 1990). As such, a certain degree of plasticity in traits associated with their migration timings are likely advantageous so that individuals can respond to local conditions (Barbee et al. 2011). The inward migrations of slower growing older post-larvae in September might be related to their ability to delay metamorphosis so they can migrate when the conditions are appropriate. Delayed metamorphosis is speculated among amphidromous species (Lord et al. 2010, Hogan et al. 2014, Lejeune et al. 2016) because of the considerable variation in age at inward migration.

However, no studies have related their developmental growth trajectory with age to confirm this mechanism.

There was no evidence for region-specific sources of growth variation among cohorts. Instead, mean daily growth rates were lowest in September and highest in late October across the four regions. The most plausible reason for this is that in migratory species, large scale environmental synchronisers acting on growth (e.g., climate) are more readily detected than subtle fluctuations in abiotic conditions (Frederiksen et al. 2014). Consequently, detecting differences in growth at two weekly intervals over thousands of kilometres is less feasible. Furthermore, region specific seasonal variation at a monthly resolution ($Month_{increment}$) accounted for most growth variation and was a more powerful metric of temporal growth variation than among cohorts.

3.5.3.3 River

The intrinsic ontogenetic growth trajectories generally showed less variation among rivers in each region. This result was unsurprising given what is known about the life history of inanga. There is little or no evidence to date that suggests inanga larvae are retained in river plumes or estuaries for a significant portion of their life that would result in river-specific differences. After a dispersive period, post-larvae return to rivers from marine larval pools in the wider area (Hickford and Schiel 2016) where the abiotic conditions are likely considerably more homogenous than freshwater environments. Significant differences were identified among rivers in the Bay of Plenty and Canterbury. Rivers within these regions differ in characteristics like catchment order, land use and source of flow (see Chapter 1), factors that are known to influence size at migration in other diadromous species (Turner and Limburg 2012) and may have an effect on growth for inanga. The factors underlying these results remain unclear, but studies over multiple years that integrate biotic and abiotic factors with growth might lend better insight into growth variation between rivers in the Bay of Plenty and Canterbury regions.

3.5.4 Growth shapes regional population dynamics

Multiple lines of evidence suggest that larvae in Buller are recruiting from a larval pool with relatively homogenous conditions. Firstly, growth among individuals was at least two times less variable than the other three regions. In addition, within-individual growth synchrony was considerably higher here and growth variation showed much stronger seasonal trends compared to the other regions. Growth among hatch months and cohorts was consistent, and no differences were found among rivers. The (mostly) uni-directional flow of the Westland

Current (Chiswell et al. 2015) is one mechanism that may explain these patterns. This is because there is less potential for inter-regional larval exchange that would obscure the relatively homogenous growth patterns found here. Furthermore, regional retention of larvae may also be enhanced by seasonal reversals of the Westland Current that occur (Menge et al. 2003). Oceanographic mechanisms likely play an important, yet little understood, role for the demographics of populations in the Buller region.

Hickford and Schiel (2016) showed that the dynamics of inanga on the east coast of the South Island are difficult to resolve. This is partially due to the hydrological complexity of the Southland Current and the potential for larval dispersal from a wide range of sources (Hickford and Schiel 2016). In this study, growth of Canterbury fish was comparatively more variable. Firstly, most of the growth variation was due to seasonal variation and differences among cohorts. The intrinsic ontogenetic component explained over 20% less growth variation in Canterbury than the ontogenetic components in each other region. It is worth nothing that error during otolith increment measurement is an important additional source of variation that is not accounted for in the analysis. Unexplained variation between fish-length and otolith-width is likely due to age or growth effects. It is known that inanga larvae aggregate at sea before migrating through estuaries and into freshwaters (McDowall 1968). Upon migration, there is little evidence of recent feeding activity and it is likely that energy is not being acquired for additional somatic growth. Therefore, although growth in terms of length has ceased (McDowall and Eldon 1980), the otolith may continue to grow laterally and also vertically. The otoliths of Canterbury larvae were noticeably more complex and variable than the relatively uniform otoliths of Bay of Plenty larvae for example. Because of their slower growth, increments were narrow and sometimes difficult to discern here and may partially explain the lower overall variation explained in Canterbury.

The growth trajectories of inanga in the Bay of Plenty and Canterbury were vastly different. Undoubtedly, this shows that larvae in each of these regions have on average spent most of their life in two distinct water masses. The growth trajectories of Golden Bay and Buller are indistinguishable which suggests considerable inter-regional larval exchange and/or that the environmental conditions in the wider regions are similar which results in relatively uniform pelagic growth rates.

3.6 Conclusions

Altogether, a wealth of information about the "black box" pelagic phase of inanga was gained from otolith microstructure methods coupled with mixed effects modelling. Importantly, this approach ties together intrinsic and extrinsic sources of growth variation into one unifying framework to describe their pelagic somatic growth histories. It is clear that disentangling sources of variability among individuals will lend important insights into inanga population dynamics because ultimately, growth and dispersal at the individual level shapes connectivity patterns among populations (Nanninga and Berumen 2014).

Chapter 4: Sagittal otolith morphology as a tool to discern population structure of an amphidromous galaxiid

Summary

The inward migrating post-larvae of inanga (*Galaxias maculatus*) are a key component of New Zealand's whitebait fishery. Knowledge of their stock structure is poorly resolved which poses problems for fisheries management. Currently, inanga are considered as one biological unit and fishery management practices do not account for any spatial or temporal variation in their life-histories, demographics or population dynamics. Here, otolith morphology was used to investigate spatial heterogeneity among populations from regions with variable oceanographic current structures and abiotic conditions. Morphological comparisons were made among autumn- and winter-hatched post-larvae because of the potentially varying dispersal abilities and origins of fish hatched at different times of the year. There were clear morphological differences among regions of New Zealand that were consistent for autumn- and winter-hatched fish. These results indicate that there are multiple regional stocks of inanga as well as zones of mixing in New Zealand and have considerable implications for the management of the fishery.

4.1 Introduction

The concept of a stock, which is defined as a semi-discrete group of fish with definable characteristics based on their genotypic, phenotypic and demographic attributes, is fundamental to the management of fisheries (Begg et al. 1999). For exploited species, knowledge of these attributes is imperative to ensure sustainable harvest, management and conservation (Midway et al. 2015). Spatial variation in vital rates such as size and age at maturity, growth rates, reproductive rate, spawning times along with immigration/emigration and mortality are critical in determining population level dynamics in fish (Secor 2007). How these parameters vary across a species distribution as well as how they intersect with fishing practices are used in stock assessments to help determine the most appropriate scale of management (Brown et al. 1987).

Determining the spatio-temporal dynamics of highly migratory species and identifying appropriate management measures is challenging (Stransky 2005). This is even more difficult

for fast-growing annual species, which are especially susceptible to overexploitation and require precise knowledge of their stock structure for fishery management (Aguera and Brophy 2011). In New Zealand, five species of amphidromous galaxiids comprise a cultural, recreational and commercial 'whitebait fishery' found throughout the country. Inanga (*Galaxias maculatus*) are considered the most important component of this multi-species fishery because of their high prevalence in the catch, up to 88% of all fish caught each year (Yungnickel 2017). This fishery is unusual because small, translucent, post-larvae (total length = 34-56 mm, 2-6 months old) are harvested during their inward migration from their marine pelagic habitat to freshwater. Adult freshwater habitats and riparian spawning habitat have been compromised throughout the country mostly because of urbanisation and agricultural intensification (Hickford and Schiel 2011, Goodman et al. 2014). However, whether fishing pressure has negative effects on populations is unknown. A further problem is that the fishery is data-limited; there is no obligation to maintain catch records, there are no quotas and there is limited population monitoring (McDowall 1996). Inanga post-larvae migrate upstream along rivers banks during a relatively predictable temporal window, so they are highly susceptible to extensive exploitation because of their relative ease of capture. Currently, the fishery is managed as two separate units; the West Coast of the South Island and the rest of New Zealand. Variations in the length of the fishing season as well as restrictions on equipment and fishing locations are the primary controls on the fishery (New Zealand Statutory Regulations 1994/65, 1994/66).

The early life history and migratory dynamics of inanga are poorly understood, especially the spatial and temporal extent of larval dispersal. This means that resolving their stock structure is difficult because simple information on their pelagic life and demographics is largely missing (McDowall 1996). The framework best describing their populations is meta-population dynamics whereby larval dispersal facilitates considerable inter-population exchange (Hickford and Schiel 2016), which to date warrants the consideration of inanga as a single biological unit (McDowall 1996). However, it is speculated that larval dispersal of inanga across oceanic basins is unlikely to be demographically relevant (Barbee and Swearer 2007) and that there is tentative evidence for regional stocks in New Zealand. McDowall and Eldon (1980) hypothesised that sub-population structures exist between the North and South Island, based on regional variation in post-larval size at inward migration. Furthermore, inanga in the north-eastern part of the North Island are significantly younger at inward migration compared to those on the west coast of the South Island (McDowall et al. 1994). There is also variation in age between the west coasts of the North and South Island (Rowe and Kelly 2009)

which shows that spatial variation in their life history traits exists. McDowall (2003), showed that inanga post-larvae in the North Island have fewer vertebrae than those in the South Island, which might be indicative of larvae having resided in thermally distinct water masses (Iguchi et al. 2006). Hickford and Schiel (2016) found little evidence of natal homing, meaning that population structuring at the river scale is unlikely. However, they found evidence for discrete larval pools based on regional variation in otolith-chemical signatures (Hickford and Schiel 2016). Conversely, mitochondrial DNA studies by Waters et al. (2000) showed extensive dispersal with little genetic structuring, which suggests inanga larvae can disperse extensively throughout New Zealand. The existence of sub-populations and/or separate stocks is one of the priorities for the management of the whitebait fishery in New Zealand (McDowall 1999) but still remains poorly resolved.

McDowall (2010) hypothesised that amphidromous species could exploit oceanographic features to minimise dispersal, but this hypothesis was not well developed. Despite widespread dispersal being largely assumed for many amphidromous species, numerous studies indicate that oceanographic features restrict dispersal or transport of larvae along different currents (Radtke et al. 2001, Iguchi et al. 2006, Lord et al. 2010, Lord et al. 2012, Warburton 2015, Hickford and Schiel 2016). From a biological perspective, adult reproductive patterns, duration of the pelagic larval stage, larval biology and recruitment dynamics can alter dispersal pathways and inter-population exchange of species with pelagic larvae (Treml et al. 2012). Coupled with oceanography, variation in spawning times is a mechanism proposed to structure larval stages in space and time (Fortier and Leggett 1983). Inanga spawning shows a latitudinal gradient in New Zealand with summer spawning mostly found in the south and winter spawning in the north (Taylor 2002, Hicks et al. 2013). Therefore, there is potential for spatially variable levels of mixing among regions according to hatching times. My previous work identified winter-hatched post-larvae in Canterbury (see Chapter 2) even though winter spawning is not widely observed in this region (Taylor 2002, Stevens et al. 2016). This suggests that these fish might have originated from elsewhere. Regional variation in age at inward migration and growth further suggests there are variable levels of spatial structuring because autumn-hatched fish have longer larval durations and spend significantly longer in the pelagic phase than do winter-hatched post-larvae.

4.1.1 Otolith morphology

Otolith morphology is one of many methods that can be used to identify and characterise stock structure (Campana and Casselman 1993). Otolith morphology can be quantified in many ways but the most used methods are landmark and elliptical Fourier analyses (Cadrin 2000). Otolith morphology is affected by genetics and an individual's environmental history like temperature and feeding rates along with intrinsic factors like sex and metabolic rate (Cardinale et al. 2004, Gagliano and McCormick 2004). Regional variation in metabolism and subsequent otolith growth rates underlie otolith shape differences among stocks (Cardinale et al. 2004). This is because individuals with different growth rates will have different otolith morphologies. Spatial and sometimes temporal differences in abiotic conditions can cause variation in otolith morphologies among populations over large spatial scales, but local environmental variation can mask these differences (Midway et al. 2014).

During early life, fish are particularly sensitive to their abiotic environment (Pepin et al. 2001). If fish encounter different environmental conditions after hatching and release into the pelagic habitat, otolith morphology can be largely influenced by their initial dispersal pathway and the conditions they encounter (Libungan et al. 2015). In addition, the relative accretion rate of otoliths is larger during early life, so younger life stages are potentially well-suited for otolith shape analysis (Hüssy 2008). Otolith morphology might be a particularly appropriate tool for studies of the population structure of inanga due to the wide range of abiotic conditions encountered during pelagic development, protracted spawning times and because inanga show significantly different metabolic rates with increasing temperatures (Milano et al. 2016), all of which can affect morphology.

4.1.2 Aims and objectives

This chapter evaluates otolith morphology as a population marker and examines its feasibility as a tool to discriminate among putative populations of inanga. The key objective was to describe and assess the integrity of populations according to hatching times from regions with varying abiotic and oceanographic conditions. I hypothesised that:

1. If inanga larvae are spending most of their pelagic phase in distinct water masses with little inter-regional exchange, then regional variation in otolith morphology will be found.

2. Inter-regional exchange varies with hatching times. Winter-hatched post-larvae might show greater regional structuring compared to autumn-hatched post-larvae because they are faster growing, spend less time as larvae and are significantly younger at inward migration, suggesting their dispersal is more restricted relative to autumn-hatched fish.

An alternative hypothesis is that considerable inter-regional larval exchange results in homogenous otolith morphologies and no regional differentiation.

4.2 Materials and methods

4.2.1 Study sites and sample collections

The oceanographic and abiotic characteristics of the four study regions (Bay of Plenty, Golden Bay, Buller and Canterbury) are described in Chapter 1. Autumn- and winter-hatched post-larvae for morphometric analysis were randomly subsampled from those that were aged in Chapters 2 and 3. Post-larvae were chosen from each river within each region to ensure a representative sample was obtained. Additional samples for morphometric analysis were taken from the pool of unaged fish. The exact hatch dates of these fish were not estimated. However, hatch date and migration date are highly correlated and there is a clear distinction in the migration timing of autumn- and winter-hatched fish between September and November (see Chapter 2). Therefore, I considered that fish migrating in September were autumn-hatched and those in November winter-hatched.

Insufficient numbers of autumn-hatched fish were available for morphometric analysis in the Bay of Plenty, and regional differences in size at inward migration occur (see Chapter 2). To minimise the effects of these on otolith morphology, the dataset of autumn- and winter-hatched fish was restricted to the size class 46-56 mm total length (L_T) for each region. Nevertheless, each region's dataset of autumn- and winter-hatched fish still contained fish of variable ages and growth rates, making them suitable for comparative analyses.

4.2.2 Image capture and morphometric measurements

For each post-larva, the left sagittal otolith was photographed medial side up (sulcus acusticus side) in a standardised orientation (Fig. 4.1). Images were taken in dark field with an AxioCamHRc CCD camera attached to a Zeiss compound microscope (Axio Imager.M1). Otoliths composed of vaterite or those that were damaged during the extraction process were

discarded. Some otoliths were concave, which created difficulties for image acquisition so were discarded. Images were imported into Image-Pro Premier v 9.1 and morphological measurements were taken of the (otolith length (O_L), otolith width (O_W), otolith perimeter (O_P) and otolith area (O_A)). These four measurements were used to calculate six shape indices: circularity, roundness, ellipticity, rectangularity, form factor and aspect ratio (Table A.4.1).

Elliptical Fourier analysis (EFA) was used to analyse the outline of each otolith (Campana and Casselman 1993). EFA quantifies outlines in terms of sine and cosine waves that measure the degree of bending or turning required to approximate the curve (Kuhl and Giardina 1982). Otolith outlines were traced in a clockwise direction using the outline macro in Image-Pro Premier v 9.1 and saved as a series of x,y coordinates. EFA was done in the R package Momocs (Bonhomme et al. 2014). Each otolith outline was centred, scaled and aligned using the first three coefficients (a1, b1 and c1) of Harmonic 1. This was done to standardise for size, orientation and starting point and to ensure that the morphometric analysis was based on the shape of the otolith and not the size (Campana and Casselman 1993). Alignment of otolith outlines was done using the Feret's diameter which aligns the otolith according to the longest ellipse. This method was used because some otoliths had no distinguishable landmark (i.e., rostrum) to align the outline using landmark-based methods.

Harmonic power was calculated to estimate the number of harmonics required to describe otolith outlines sufficiently. Lower order harmonics describe gross morphology such as circularity and elongatedness, while higher order harmonics reflect more localised and detailed changes (Campana and Casselman 1993). Harmonic power quantifies the complexity of the otolith but also ensures that the number of harmonics used can be reduced for multivariate analysis without losing important information about otolith morphology.

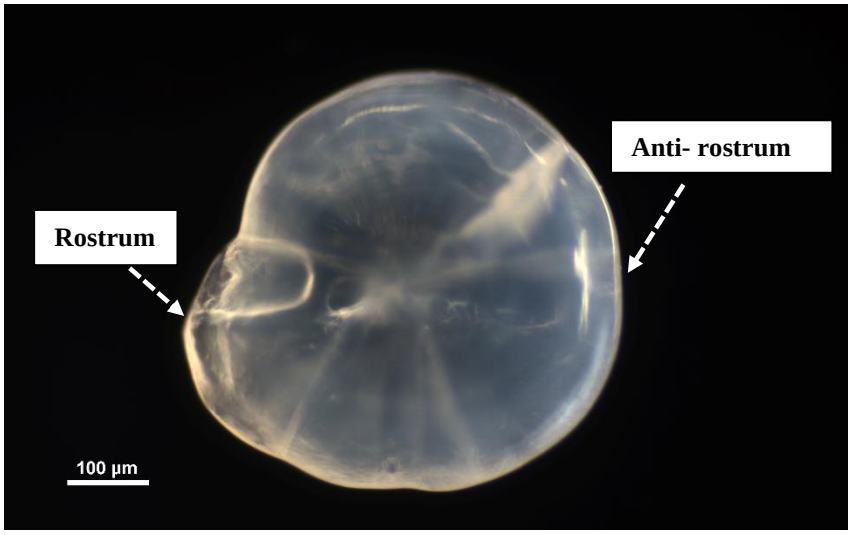


Figure 4.1. Example of an otolith used for morphological analysis.

4.3 Data analysis

Kolmogorov-Smirnov (K-S) tests were done to test for differences in the length-frequency distribution of autumn- and winter-hatched post-larvae among regions. ANOVA was used to test for differences in mean L_T of post-larvae among regions.

ANCOVA was used to test for the effects of otolith length ($O_{L)}$ on the six shape indices with 'region' as a factor to examine regional variability in these measurements. If the interaction between region and size was significant, this meant that the slope of the relationship with the shape-variable was not constant among regions. In this event, the shape variable in question was removed from the analysis (Aguera and Brophy 2011). Shape indices that were significantly correlated with the size measurements were corrected for size-effects using the common within-group slope (b) from the ANCOVA model. To do this, the product of the slope and otolith-size relationship was subtracted from the shape variable using the formula

$$Yc = Y - b \times O_L$$

where Y_c is the size corrected shape variable, Y is the original shape variable, b is the common within-group slope value and O_L is otolith length (Burke et al. 2008).

One-way ANOVA tested for regional variation in shape variables and EFCs. Tukey's honest significance difference (HSD) post-hoc comparisons were done to identify significant differences among regions. Multiple comparisons among regions were Bonferroni-corrected and significance reported as 95% confidence intervals. Shape indices and EFCs that were significantly different among regions were selected for multivariate analysis to increase discrimination and reclassification rates (Aguera and Brophy 2011).

Principal components analysis (PCA) was done on the significant shape descriptors and EFCs and was based on the correlation matrix. PCA was used because it reduces the number of variables describing otolith morphology into independent, orthogonal predictors that systematically summarise variability in otolith shape (Strauss 2010). Scree plots (eigenvalues against PC number) were used to identify the number of principal components needed to capture most variation in otolith morphology for multivariate analysis. PCs with cumulative explained variation of 99% were chosen for further analysis.

Linear discriminant function analysis (DFA) was used as a classification method used to discriminate regional groupings of autumn- and winter-hatched post-larvae. PCA scores were entered into the DFA model in a stepwise manner and only PCs that were significant at $\alpha = 0.01$ were used in the DFA. The probability of group membership for autumn and winter-hatched fish was allowed to vary among regions according to the sample sizes used. Wilks lambda (λ) was used to evaluate discriminatory power from the results of the DFA. Jack-knifed reclassification was used as a cross-validation procedure to assign fish back to their hypothetical regional populations. DFA scores for autumn- and winter-hatched post-larvae were plotted and the centroid calculated for each region.

For the ANCOVAs and ANOVAs, shape-indices and EFCs were tested for normality using Shapiro- Wilks tests and heterogeneity of variance using Levene's tests. Transformations were done (square root, log and arcsine) if necessary to stabilise normality and variance heterogeneity. Variables that could not be successfully transformed to meet these statistical assumptions were removed from the analysis. The final set of PCA scores were examined for normality and variance heterogeneity before being used in the DFA.

All statistical analyses were performed in R 3.2.2 (R Core Development Team 2015). Elliptical Fourier analysis was done using the momocs package (Bonhomme et al. 2014). PCA and DFA were done in the MASS package (Venables and Ripley 2002).

4.4 Results

4.4.1 Image acquisition

The otolith morphologies of 200 autumn- and 157 winter-hatched post-larvae were successfully quantified. In general, image acquisition was easy, with little outline aberration. Nine autumn- and six winter-hatched otoliths were discarded from the total dataset because there were either irregularities in their outlines, most likely a result of damage during otolith extraction, and

significant outliers were found during the data analysis. This resulted in 191 autumn- and 151 winter-hatched otoliths for analysis (Table 4.1).

4.4.2 Size

For autumn-hatched fish, KS-tests showed no significant difference in the distribution of post-larval L_T among regions (Table A.4.2, Fig. A.4.1) and no differences in mean L_T were found among regions (ANOVA $F_{2,188} = 3.02$, $p > 0.05$). Winter-hatched post-larvae showed significant differences in L_T distributions among regions (Table A.4.2, Fig. A.4.1) as well as differences in mean L_T (ANOVA $F_{3,147} = 30.5$, $p < 0.001$). Winter-hatched post-larvae in Bay of Plenty were significantly smaller than those in Golden Bay, Buller and Canterbury and post-larvae in Canterbury were smaller than Buller (Table 4.2).

4.4.3 Shape indices

Spearman's correlations showed strong correlations among shape indices for autumn- and winter-hatched post-larvae (Table A.4.3). Form factor and circularity were removed from each hatch season's dataset because they were not normally distributed (Shapiro Wilks, $p < 0.01$), had heterogeneous variances (Levene's, $p < 0.01$) and could not be appropriately transformed. Rectangularity was log-transformed for autumn-hatched post-larvae, but not winter-hatched. For both hatch-seasons, roundness, ellipticity and aspect ratio were normally distributed with homogenous variances and no transformation was required.

Table 4.1. Total length (L_T) characteristics of autumn- and winter-hatched post-larvae among regions that were used for otolith morphometric analysis.

	Autumn-hatched				Winter-hatched			
Region	**n**	**Dates**	**L_T range**	**Mean L_T ± 1.SD**	**n**	**Dates**	**L_T range**	**Mean L_T ± 1.SD**
Bay of Plenty					39	Sep 2013	46.90 – 52.5	49.20 (1.58)
Golden Bay	64	Sep 2013	49.57 – 55.84	52.31 (1.61)	38	Nov 2013	47.18 – 55.9	52.41 (1.89)
Buller	64	Sep 2013	49.32 – 55.74	52.98 (1.32)	38	Nov 2013	46.70 – 55.9	53.26 (1.85)
Canterbury	63	Sep 2013	48.25 – 55.95	52.76 (1.74)	36	Nov 2013	47.30 – 55.8	51.74 (2.48)

Table 4.2. Bonferroni corrected 95% confidence intervals of p-values for multiple comparison tests among regions for differences in total length (LT) of winter-hatched post-larvae. Intervals that overlap zero indicate comparisons that are not significant.

Regions	**Estimate**	**95% CI**
Bay of Plenty – Golden Bay	3.21	2.04 – 4.38
Buller – Bay of Plenty	4.07	2.90 – 5.23
Canterbury – Bay of Plenty	2.54	1.35 – 3.72
Buller – Golden Bay	0.86	-0.32 – 2.03
Canterbury – Golden Bay	-0.67	-1.86 – 0.52
Canterbury – Buller	-1.53	-2.72 – 0.34

ANCOVA results for each hatch-season showed the interaction term between region and otolith length (O_L) for each shape variable was not significant (Table A.4.4). Pearson's correlations showed that O_L was significantly correlated with each shape index except rectangularity for winter-hatched post-larvae (Table 4.3). Size correction was done (using the common within-group slope from the ANCOVA for the shape indices that were significantly correlated with O_L (Table A.4.4).

Table 4.3. Pearson's correlations between otolith length (OL) with each of the shape indices for autumn- and winter-hatched post-larvae. Significant correlations are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS denotes non-significant correlation.

Hatch-season	Shape indices	r	p
Autumn	Rectangularity	-0.21	**
	Roundness	-0.46	***
	Aspect ratio	0.44	**
	Ellipticity	0.44	***
Winter	Rectangularity	-0.13	NS
	Roundness	-0.26	***
	Aspect ratio	0.23	**
	Ellipticity	0.23	**

The shape indices aspect ratio and ellipticity were significantly different among regions for autumn- and winter-hatched post-larvae (Table 4.4). Roundness was significantly different among regions for winter- but not autumn-hatched post-larvae (ANOVA $F_{2,188} = 2.57$, $p > 0.05$, Table 4.4). Rectangularity was not significantly different for autumn- (ANOVA $F_{2,188} = 2.05$, $p > 0.1$) or winter-hatched post-larvae (ANOVA $F_{3,147} = 0.27$, $p > 0.5$). Altogether, two shape indices for autumn- and three for winter-hatched post-larvae were considered for inclusion in the principal components analysis.

Table 4.4. ANOVA results for significant differences in shape indices of autumn- and winter-hatched post-larvae among regions. Significant differences are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Hatch-season	Shape indices	F	df	p
Autumn	Aspect ratio	6.74	2,188	**
	Ellipticity	6.75	2,188	**
Winter	Aspect ratio	7.87	3,147	***
	Ellipticity	7.47	3,147	***
	Roundness	9.83	3,147	***

4.4.4 Elliptical Fourier analysis

The Fourier power equation showed that 99% of the variation in otolith outlines was captured by 9 harmonics with 36 EFCs. The standardised coefficients of harmonics 1 (a1, b1 and c1) were not used in the analysis. This left 33 EFCs for further analysis of outline morphology. For autumn-hatched post-larvae, seven EFCs showed significant differences among regions. These were A5, A8, B6, B7, C5, C8, and D3 (Table 4.5). For winter-hatched post-larvae, only B4 and B6 showed significant differences among regions (Table 4.5).

Table 4.5. ANOVA results of Elliptical Fourier coefficients (EFC) that showed significant differences among regions for autumn- and winter-hatched post-larvae. Significant differences are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Hatch-season	EFC	F	df	p
Autumn	A5	6.43	2,188	**
	A8	3.74		**
	B6	4.82		**
	B7	7.72		***
	C5	6.97		**
	C8	3.40		*
	D3	8.81		***
Winter	B4	2.88	3,147	*
	B6	2.93		*

4.4.5 Principal components analysis

4.4.5.1 Autumn-hatched

For autumn-hatched post-larvae, 99% of the variance in otolith morphology was explained by seven principal components (PC). EFCs B7 and C5 made the strongest negative contributions and B6 and D3 made positive contributions to PC1 (Fig. 4.2, Table A.4.5). On PC2, the shape indices made the greatest positive contribution while C8 made the strongest negative contribution (Fig. 4.2, Table A.4.5). The cumulative amount of variance explained by the first two PCs was 67.7% (Fig. 4.2).

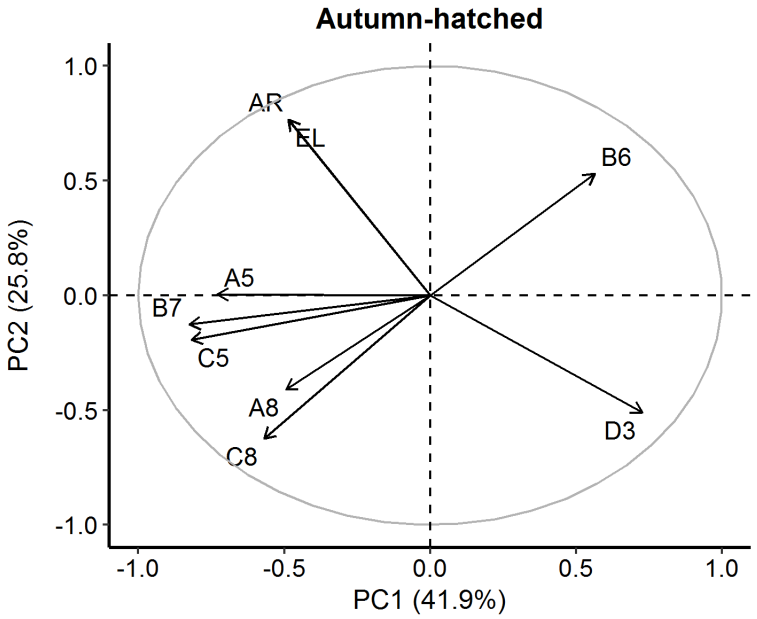


Figure 4.2. Bi-plot showing the loadings of the significant shape indices and Elliptical Fourier coefficients for the first two principal components for autumn-hatched post-larvae.

4.4.5.2 Winter-hatched

For winter-hatched post-larvae, the shape indices aspect ratio and ellipticity made the strongest positive contributions to PC1 while roundness contributed negatively to PC1 (Fig. 4.3, Table A.4.5). On PC2 the EFCs made the strongest positive contributions (Fig. 4.3, Table A.4.5). The cumulative amount of variance explained by the first two PCs was 85.2% (Fig. 4.3).

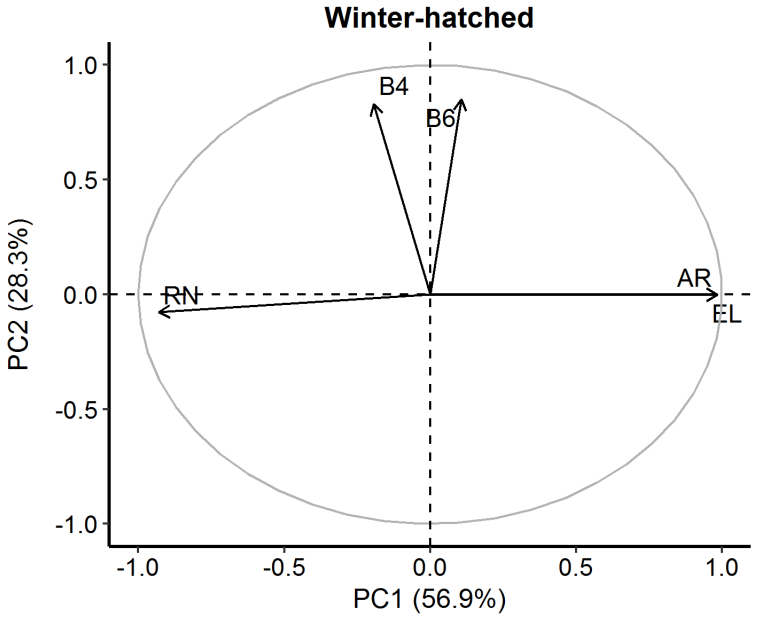


Figure 4.3. Bi-plot showing the loadings of the significant shape indices and Elliptical Fourier coefficients for the first two principal components for autumn-hatched post-larvae.

4.4.6 Linear discriminant function analysis

4.4.6.1 Autumn-hatched

PC1, PC4, PC2 and PC5 were entered into the model using forward stepwise analysis (Table A.4.6). The first discriminant axis accounted for 74% of the variance in otolith morphology and significantly discriminated among regions ($\lambda = 0.82$, $p < 0.001$). The second discrimination axis was also significant ($\lambda = 0.94$, $p < 0.05$) and accounted for 26% of the variance. The largest contribution to regional discrimination on the first axis was made by PC1 (Table 4.6). PC4 had the largest effect on the second discriminant axis (Table 4.6). The morphologies of autumn-hatched post-larvae in Canterbury were significantly different from both Golden Bay ($F_{4,185} = 6.22$, $p < 0.001$) and Buller ($F_{4,185} = 5.64$, $p < 0.001$). However, otolith morphology was not significantly different between Golden Bay and Buller ($F_{4,185} = 2.49$, $p > 0.05$) and plots of the discriminant functions did not show a distinct separation between these regions (Fig. 4.4).

The jack-knife reclassification scores showed that the percentage of correctly reclassified autumn-hatched post-larvae back to their hypothetical region of origin was 53%. The highest classification scores were for Canterbury (59%) and the lowest for Golden Bay (47%). Buller had a reclassification score of 53%. There was equal spread of misclassified fish among regions (Table 4.7).

Table 4.6. Standardised coefficients of the canonical discriminant function showing the relative contribution of the principal components (PC) to the discrimination of autumn- and winter-hatched post-larvae among regions. The PCs are ranked according to order of entry into the discriminant function analysis using forward stepwise selection.

Hatch-season	Principal component	Root 1	Root 2
Autumn	PC1	0.96	-0.23
	PC4	-0.08	-0.65
	PC2	-0.21	-0.56
	PC5	-0.18	-0.47
	Total	0.74	1.0
Winter	PC1	0.89	-0.37
	PC2	-0.41	-0.91
	PC4	0.35	-0.04
	Total	0.81	0.98

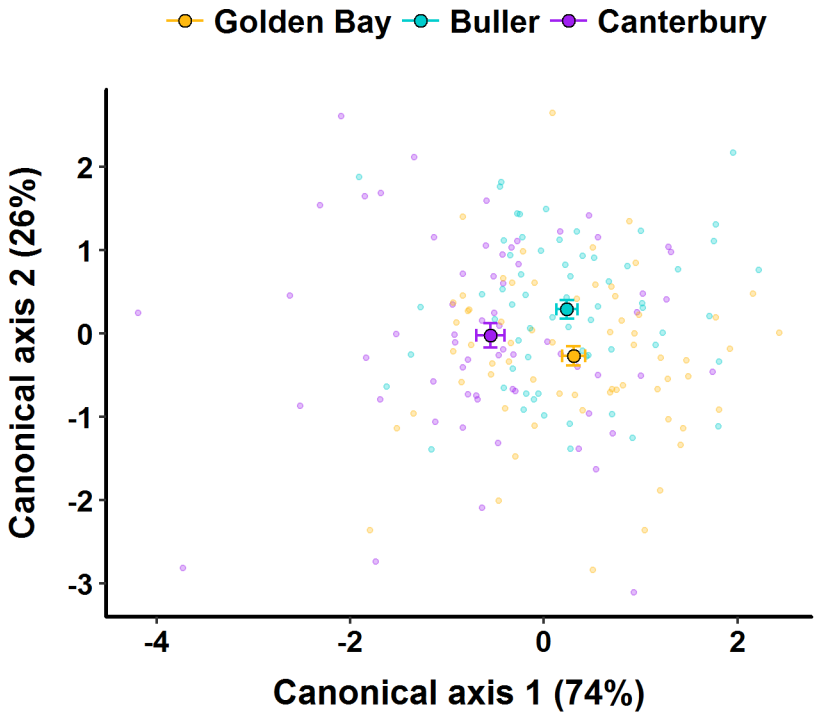


Figure 4.4. Linear discriminant analysis of otolith morphology for autumn-hatched post-larvae among regions. The larger circles are the group centroids and the error bars are the standard errors of the mean.

Table 4.7. Jack-knife reclassification matrix from the discriminant function analysis for autumn- and winter-hatched post-larvae among regions.

	Origin	**Predicted origin**			
Hatch-season	**Region**	**Bay of Plenty**	**Golden Bay**	**Buller**	**Canterbury**
Autumn	Golden Bay (n = 64)		32	14	18
	Buller (n = 64)		17	35	12
	Canterbury (n = 63)		14	13	36
	Total		63	62	66
Winter	Bay of Plenty (n =39)	22	6	7	4
	Golden Bay (n = 38)	9	10	13	6
	Buller (n =38)	5	5	26	2
	Canterbury (n =36)	16	8	5	7
	Total	52	29	51	19

4.4.6.2 Winter-hatched

PC1, PC2 and PC4 were entered in the DFA model, with PC1 and PC2 contributing significantly to the discrimination of winter-hatched post-larvae among regions (Table A.4.6). PC1 had the largest contribution to group differentiation on the first axis and PC2 on the second (Table 4.6). The first discriminant axis accounted for 81% of the variance in otolith morphology of winter-hatched post-larvae and successfully discriminated among regions (λ = 0.76, $p < 0.001$, Fig. 4.5). The second axis accounted for 17% of the variation but showed no significant discriminatory power ($\lambda = 0.94$, $p > 0.05$).

Winter-hatched post-larvae in the Bay of Plenty had significantly different otolith morphologies compared to Golden Bay ($F_{3,145} = 4.55$, $p < 0.01$) and Buller ($F_{3,145} = 9.05$, $p < 0.001$) but not Canterbury ($F_{3,145} = 0.77$, $p > 0.5$). Winter-hatched Canterbury post-larvae were significantly different to Buller ($F_{3,145} = 8.17$, $p < 0.001$) but only showed weak differences compared to Golden Bay ($F_{3,145} = 2.66$, $p < 0.05$). Differences between Buller and Golden Bay were significant but weak ($F_{3,145} = 3.45$, $p < 0.05$).

The average jack-knife reclassification success to region of origin was 43%, but substantial regional variation was found (Table 4.7). The highest reclassification success was 68% for Buller and the lowest in Canterbury at 19%. The reclassification success for Golden Bay was 26% and Bay of Plenty was 56%. Plots of the discriminant functions showed that the group centroids for Bay of Plenty and Canterbury were clearly separated from Golden Bay and Buller but among all regions there was a small number of individuals that overlapped each region's distribution of points (Fig. 4.5).

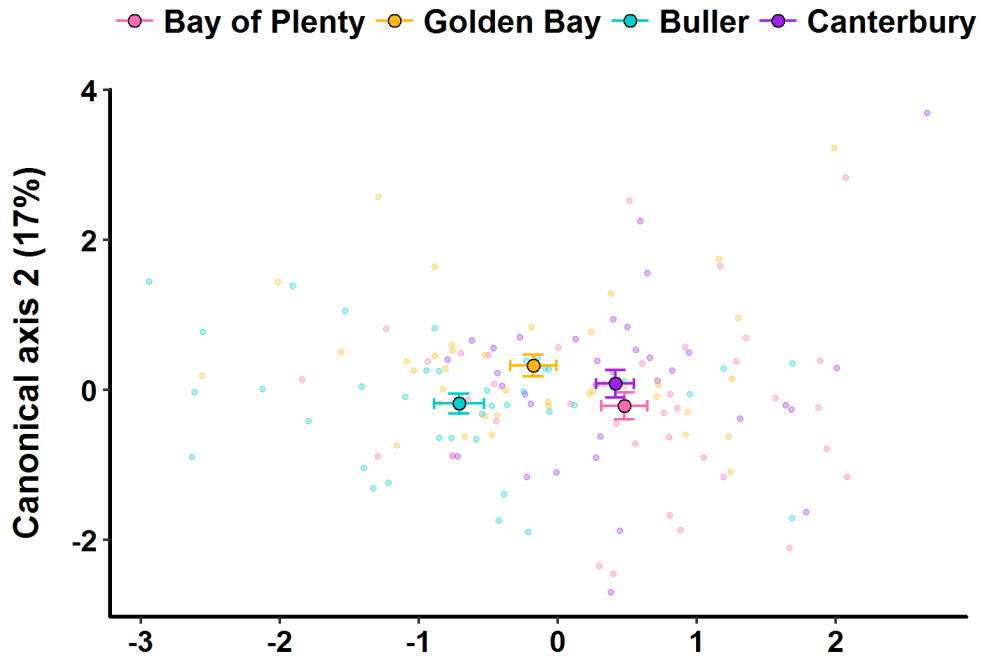


Figure 4.5. Linear discriminant analysis showing the discrimination of winter-hatched post-larvae among regions. The larger circles are the group centroids and the error bars are the standard errors of the mean.

4.5 Discussion

In this study, the otolith morphologies of autumn- and winter-hatched inanga post-larvae were characterised among regions. This information was used to investigate the integrity of putative regional populations. Morphological comparisons showed significant regional differences for autumn- and winter-hatched post-larvae. Greater spatial structuring of winter- compared to

autumn-hatched post-larvae was found, suggesting that regional integrity varies as a function of hatch-season. The overall reclassification rates back to each putative population were over 43% and varied among regions. Overall, otolith morphology seems to be a good indicator of the growing environment. The regional variations suggest that some regions are mixing zones where post-larvae originate from multiple populations whereas other regions show a more discrete population structure indicative of little inter-population exchange. These results provide the first insights into the population structure of inanga using a morphological based approach.

4.5.1 Otolith morphology

Elliptical Fourier Analysis (EFA) was used to reconstruct otolith outlines of inanga while shape indices were derived from a suite of otolith-size based measurements. The EFA showed that inanga have a relatively simple otolith morphology because only 9 harmonics were required to quantify the outline. In contrast, up to 40 harmonics are required for species with convoluted and lobed otoliths like Atlantic cod (Galley et al. 2006). Higher order harmonics showed significant regional differences for autumn- and winter-hatched post-larvae, indicating there are larger- rather than finer-scale differences in otolith morphologies (Paul et al. 2013). Although the proximate factors influencing otolith morphology were not directly examined here, it is likely that spatial and temporal variation in the abiotic conditions encountered during pelagic development was a driver, shown by autumn-hatched post-larvae having had more variable and complex otolith morphologies compared to winter-hatched post-larvae. One potential reason for this is that autumn-hatched post-larvae spend significantly longer in the pelagic environment and are older at migration (see Chapter 2 and 3). This means they likely experience a much broader range of abiotic conditions than winter-hatched post-larvae that spend significantly less time in the pelagic. Additionally, autumn-hatched post-larvae showed greater regional variation in otolith outline morphology whereas winter-hatched post-larvae were mostly discriminated by shape indices. Winter-hatched fish are faster growing and so the relative accretion rates in their otoliths are larger (Hüssy 2008), which might explain why shape indices, and not the Elliptical Fourier coefficients (EFCs), were more variable for winter- compared to autumn-hatched post-larvae.

4.5.2 Regional variation in morphology is a function of the physical and environmental conditions in the pelagic environment

Regional variation in otolith morphology was largely consistent with broad scale oceanographic patterns. Winter-hatched post-larvae in the Bay of Plenty and Buller showed the greatest distinction in otolith morphologies. This is consistent with empirical and modelling studies for other taxa. For example, dispersal of simulated propagules by Chiswell and Rickard (2011) showed that propagules arriving into Tauranga Harbour in the Bay of Plenty and Westport Harbour in Buller mostly originated from within the wider region, with no evidence of propagule exchange between the two harbours. Studies of molluscs and bivalves with pelagic dispersal stages consistently show genetic differentiation between populations on the north-eastern coast of the North Island and the west coast of New Zealand (Apte and Gardner 2002, Waters and Roy 2004, Ross et al. 2009). Furthermore, Warburton (2015) found significant genetic structuring between populations north of the Bay of Plenty (sampling was not done in Bay of Plenty but in the Firth of Thames) and Buller for the amphidromous torrentfish (*Cheimarrichthys fosteri*) using mitochondrial DNA analyses. Whether the morphological differences between Bay of Plenty and Buller are driven by environmentally induced phenotypic differences, genetic differences or the interaction of both factors is uncertain. Inanga are considered panmictic with extensive larval dispersal occurring throughout all of New Zealand (Waters et al. 2000), so genetic differentiation among regions is less likely. However, Waters et al. (2000) results must be interpreted with caution as older techniques were used that cannot readily discern population structure. The morphological differences may be better explained by significant differences in the pelagic growth trajectories of winter-hatched post-larvae between these regions (see Chapter 3), along with differences in age at inward migration as well migration date (as in Chapter 2) which are likely mediated by regional variation in sea surface temperature and productivity.

The otolith morphology of winter-hatched post-larvae in Buller showed the highest reclassification rates back to this region (68% success). The reclassification of autumn-hatched post-larvae back to Buller was moderate (53% success). These results correspond well with the growth reconstructions in Chapter 3 whereby the growth trajectories of post-larvae in Buller were the most uniform among the studied regions. I previously hypothesised that post-larvae in Buller are recruiting from a relatively homogenous larval pool and that the (mostly) uni-directional flow of the Westland current restricts inter-regional larval exchange (Chapter 3). The morphometric analyses mostly support this hypothesis and other studies further indicate

that west coast populations of inanga and other amphidromous species are relatively discrete (Warburton 2015, Hickford and Schiel 2016). In Buller, seasonal reversals of the Westland Current occur that are inter-related with SST and upwelling/downwelling systems (Menge et al. 2003). These reversals might enhance regional retention of larvae hatched at different times of the year. Such mechanisms are responsible for the spatial and temporal patterns found for other species with pelagic larvae and protracted spawning seasons (Bruce et al. 2001, Underwood et al. 2012) and might explain the different reclassification successes between hatch-seasons that were found here. The reclassification success of winter-hatched post-larvae to Bay of Plenty was moderate showing that the integrity of winter-hatched post-larvae here is weaker than for Buller. Waters et al. (2000) suggest that some inanga in the north-east of the North Island originate from Australia because they found evidence of a trans-Tasman haplotype here. Therefore, some of the morphological variation found in the Bay of Plenty might be related to dispersal and exchange between Australian and New Zealand populations but also from populations along the eastern coast of New Zealand (Chiswell and Rickard 2011).

Post-larvae in Golden Bay consistently showed the greatest variation in otolith morphology and had the lowest reclassification success, likely reflecting the hydrological complexity of this area. Golden Bay is influenced by the northward flowing Westland Current and the D'Urville Current (Chiswell and Rickard 2011) meaning this region is likely a zone of high larval mixing from different sources. The strong tides here suggest retention of inanga larvae within the wider bay might be minimal (Heath 1985), but future studies need to confirm this. The lack of discrimination in morphologies between Golden Bay and Buller supports my previous work. In Chapter 3, I showed that the growth trajectories of post-larvae in Buller and Golden Bay were mostly indistinguishable. This indicates that post-larvae share similar environmental histories or that there is substantial exchange between these neighbouring regions. In fact, it is likely a combination of both factors because of the large influence of the Westland Current in the Golden Bay region along with similar abiotic conditions between regions (see Chapter 1).

Both autumn- and winter-hatched post-larvae in Golden Bay and Buller had significantly different morphologies from Canterbury. The growth trajectories of post-larvae in Canterbury showed no differences in the early larval stages, regardless of hatch-season. However, Canterbury post-larvae were slower growing at approximately two months prior to inward migration compared to Golden Bay and Buller (see Chapter 3). The morphological discrimination is likely related to these differences in growth trajectories, which are related to the colder and less productive conditions in the Canterbury region compared to Golden Bay

and Buller. There is potential for inter-mixing from the west coast to the east coast of New Zealand (Hickford and Schiel 2016) via the southward flowing branch of the Westland current, but this exchange is probably minimal (Chiswell and Rickard 2011). The discriminant analysis showed that some individuals in Buller were more similar to Canterbury and vice versa, meaning there was no absolute distinct separation of all post-larvae caught here. Whether this is indicative of inter-regional larval exchange is uncertain.

Winter spawning of inanga is not widely found in the Canterbury region (Taylor 2002, Stevens et al. 2016) and so the origins of winter-hatched post-larvae here are uncertain. The reclassification success of winter-hatched post-larvae back to Canterbury was 40% lower than autumn, suggesting that winter-hatched fish were more likely to have originated from elsewhere. It is unlikely winter-hatched fish originated from further south because winter-spawning is minimal in the south (Hicks et al. 2013). Surprisingly, the morphologies of winter-hatched post-larvae in Bay of Plenty and Canterbury did not differ even though significant differences in age at inward migration were found (Chapter 2) and the growth trajectories of post-larvae in these regions showed the greatest differences (Fig. 3.7). The lack of variation in otolith morphology among regions could be related to a high level of inter-population mixing. Alternatively, this result may suggest that is little mixing and instead, the proximate factors influencing otolith morphology show broad-scale similarities between regions resulting in weak discriminatory power (Hamer et al. 2012).

Temperature and food availability are two of the most important factors influencing otolith morphology in many species (Cardinale et al. 2004, Gagliano and McCormick 2004), and these factors are vastly different between Bay of Plenty and Canterbury. For example, mean winter sea surface temperatures differ by 6 degrees (see Chapter 1). Furthermore, Bay of Plenty is influenced by occasional nutrient-rich, highly productive upwelling but Canterbury is dominated by nutrient-poor downwelling (Murphy et al. 2001, Schiel 2004). The lack of morphological differentiation between these regions is therefore unlikely to be related to similar environmental histories during pelagic larval dispersal. Alternatively, local-scale variation in abiotic conditions experienced by winter-hatched post-larvae may result in highly variable growth patterns and thereby otolith morphologies. Consequently, within region variability in otolith morphology may confound the detection of large scale geographic patterns (Midway et al. 2014). The microstructure patterns of Canterbury post-larvae were more complex than elsewhere which likely reflects the highly variable conditions experienced by inanga there. Growth reconstructions in Chapter 3 showed that most of the growth variation in Canterbury and Bay of Plenty post-larvae was related to underlying seasonal trends, inter-

individual differences as well as differences among fish migrating early or later in the season. Additionally, Hickford and Schiel (2016) found that the otolith chemistry of inanga in Canterbury was highly variable, which further shows that conditions in this region are dynamic. Nevertheless, local scale variation still does not explain the lack of morphological differences among Bay of Plenty and Canterbury.

A more plausible hypothesis is that the lack of discrimination between winter-hatched Bay of Plenty and Canterbury post-larvae is due to extensive mixing along the east coast of New Zealand. Dispersal simulation studies by Chiswell and Rickard (2011) provide evidence for a northerly to southerly flow of propagules to the Canterbury region but also show a southerly to northerly flow to the Bay of Plenty. They estimated that 56% of all propagules arriving into Lyttelton Harbour in the Canterbury region originated from Wellington Harbour, with additional sources from other North Island areas such as Gisborne and Napier as well as Picton from the top of the South Island (Chiswell and Rickard 2011). Of propagules arriving into Tauranga Harbour in the Bay of Plenty, their model showed that 21% of them could originate from Gisborne with additional sources from Wellington and Napier (approx. 9%) (Chiswell and Rickard 2011). Furthermore, Warburton (2015) recently showed no genetic differentiation among amphidromous torrentfish (*C. fosteri*) populations along the east coast of New Zealand. These patterns further substantiate inter-regional exchange of pelagic larvae occurs along the east coast of New Zealand. It is possible that winter-hatched post-larvae in Canterbury and Bay of Plenty share a common origin, potentially located between Wellington and Gisborne.

4.5.3 Is otolith morphology representative of different stocks of inanga?

It is important to consider whether spatial heterogeneity in otolith morphology means there are different stocks of inanga in New Zealand. In this study, regional populations of inanga were grouped *a priori* for analysis and reclassified back to their hypothetical regions of origin. This approach therefore assumes that inanga originated from within each of the studied regions. In other studies of stock structure, baseline information is often available to warrant such an approach. For example, Brophy et al. (2016) had prior knowledge of regional variation in the genetic structure of Atlantic bluefin tuna (*Thunnus thynnus*) populations. They used this information to characterise the otolith morphologies of genetically discrete eastern and western stocks and could then estimate the relative contribution of these regional stocks to mixed aggregations. In other instances, inferences about the structure of populations and their regional fidelity are made based on dispersal simulation studies, regional variation in oceanography,

mark-recapture studies, meristics, parasite prevalence, hatch-date distributions or growth patterns (Bruce et al. 2001, Brophy and Danilowicz 2002, Burke et al. 2008). Previous studies have indicated regional variation in the population structure of inanga from meristic studies, variation in size and age at migration and otolith chemistry (McDowall and Eldon 1980, McDowall et al. 1994, McDowall 2003, Hickford and Schiel 2016). Growth reconstructions done in Chapter 3 further suggest significant variation in early life histories and phenotypic characteristics across the studied regions. Although the exact origins of post-larvae cannot be ascertained in this study, the distinct regional differences found, along with the variable reclassification rates, show that inanga display spatially complex characteristics.

The overall reclassification rates of autumn- and winter-hatched post-larvae back to their putative regions was lower than found in other studies. Libungan et al. (2015) showed that Norwegian spring-spawning Atlantic herring (*Clupea harengus*) and summer-spawning Icelandic populations could be discriminated to their feeding grounds with 94% accuracy. In Atlantic saury (*Scomberesox saurus saurus*), 86% reclassification was found between spring-spawned Atlantic populations and autumn-spawned Mediterranean samples (Aguera and Brophy 2011). Jónsdóttir et al. (2006) found 0 – 44% reclassification success among groups of spawning cod (*Gadus morhua*) in Iceland. Although reclassification was low, most cod were reclassified back to neighbouring populations and the discriminant analysis showed a clear separation of otolith morphologies among locations. The authors concluded there was sufficient evidence for two separate populations (Jónsdóttir et al. 2006). In morphological studies, discrimination and reclassification success are highly influenced by samples sizes (Strauss 2010). The samples sizes used in my study were well within the range used in other otolith morphometric studies (Aguera and Brophy 2011, Keating et al. 2014). As well, the number of post-larvae analysed was at least five times larger than the number of variables used in the discriminant function analysis (Strauss 2010) and so the analysis is considered robust. Allometric variation can confound the use of morphometric methods to discriminate among groups (Strauss 2010). The regional morphological differences found for winter-hatched post-larvae might be confounded by significant differences in L_T found among some regions. However, these differences are not considered to be a significant source of error. First, only the post-larval stages were used so all fish analysed were at the same developmental stage irrespective of differences in L_T. Second, there was no evidence of significant residual correlation with O_L for any of the shape indices or EFCs, so any effects of size variation were removed for winter-hatched post-larvae.

Based on the definition of a stock by Begg et al. (1999), which is defined as a "semi-discrete group of fish with definable genotypic, phenotypic and demographic attributes", my study shows sufficient evidence for multiple stocks of inanga in New Zealand. At the very least, populations on the west coast of the South Island, and along the east coast of New Zealand should be considered as two separate stocks. Furthermore, given there were significant differences in age and growth rates of winter-hatched fish among Bay of Plenty and Canterbury (see Chapter 2 and 3), yet no morphological differences were detected, more research is needed to ascertain if a third unit should be defined between Canterbury and Bay of Plenty. Golden Bay was identified as a potential zone of high mixing which needs to be taken into consideration for fishery management. Such mixing zones can be highly susceptible to strong fluctuations in abundance of post-larvae recruiting to the fishery due to processes occurring elsewhere in New Zealand.

To further evaluate if the patterns found are representative of the studied region, it is best if studies can be done over multiple years, although this is often difficult to achieve. Changes in the magnitude and direction of winds, especially southerlies, can have a significant effect on dispersal in other pelagic species along the west coast of the North Island (Salinas-de-Leon et al. 2012). Additionally, variation in tidal current flows can affect dispersal of species on the north-eastern coast of New Zealand (Stephens et al. 2006). These physical factors, along with intra-annual variation in abiotic conditions, especially temperature, can affect the growth, mortality and survival of larvae modifying the spatial and temporal extent of their dispersal and thereby inter-mixing. Additionally, variation in reproductive output of adults and egg mortality that can vary inter-annually can also affect dispersal because greater numbers of larvae entering the pelagic increase the probability of longer dispersal distances and thereby inter-population exchange (Duarte and Alcaraz 1989, Lester and Ruttenberg 2005). Finally, morphological differences between individual rivers within regions were not investigated. Otolith morphology often performs better in studies over large geographic scales because detecting the effects of local-scale variation on morphological features can be difficult (Stransky 2005). Other methods, like otolith microchemistry coupled with morphology can address some of these questions to better understand fine scale resolution of this species population structure. It should also be noted that a changing climate may well affect seawater temperatures, wind fields, rainfall (and therefore river flows) and numerous other factors that influence these stocks highlighting the need for studies across multiple years (Walter et al. 2012).

4.6 Conclusions

Regional larval pools and areas of mixing were identified using otolith morphological analysis with at least two stocks of inanga identified. The physical and environmental conditions of the dispersal environment can restrict dispersal and careful consideration of inanga stocks, and their management must be used to better manage the fishery. The existence of finer-scale population structure (i.e. among individual rivers) was not specifically examined in this study. However, it is recommended that future studies should investigate whether such local structuring of populations exists. Such studies likely require more refined methods than otolith morphology.

Chapter 5: Disentangling the life-history of an amphidromous fish using somatic growth reconstructions from adults

Summary

Amphidromous species, like inanga (*Galaxias maculatus*), occupy pelagic habitats during their larval life and freshwater habitats for adult growth and sexual maturity. Most studies to date examine discrete parts of amphidromous life cycles (i.e., pelagic or adult phases). Here, I took an integrative approach and reconstructed the growth trajectories of inanga adults from their larval stages through to sexual maturity. Growth was compared among hatch-seasons, and by maturity stages and sex to understand underlying drivers of variation. Size and age at maturity, along with reproductive investment, were investigated in relation to their growth histories. It was found that pelagic and adult growth were uncoupled: winter- and spring-hatched fish with faster pelagic growth rates were slower growing for their age in rivers. Autumn-hatched fish with slower pelagic growth rates maintained higher growth rates for their age as adults in the freshwater habitat. There were complex relationships between these varying growth histories and demographic characteristics (size/age at maturity and reproductive investment). This study provides some of the first critical insights into the relationships between pelagic and freshwater growth in an amphidromous species, and how this affects the population dynamics of inanga.

5.1 Introduction

Understanding the patterns and processes underlying the life histories of species is a central goal in studies of population dynamics and demographics (Braun and Reynolds 2014). For species that traverse multiple environments to complete their life cycle, like diadromous fishes (Myers 1949), their marine-freshwater migrations is one of the most fundamental aspects of their ecology. Amphidromy is a distinct diadromous migration whereby larvae develop in a pelagic habitat (mostly in the marine environment but sometimes in freshwater), but adults grow and mature in freshwater (McDowall 2010). Compared to other diadromous life histories (i.e., anadromy or catadromy), little is known about the ecology and population dynamics of amphidromous species (Closs and Warburton 2016). One reason for the lack of information is that whole life-history studies, incorporating various aspects of pelagic and freshwater life, are hindered by pelagic larval development because often the larvae are too small to locate or even

identify (Hickford and Schiel 2003, Closs and Warburton 2016). As in many species with bipartite life cycles, there is evidence that larval experiences can influence juvenile and adult characteristics (Shima and Findlay 2002, Jonsson and Jonsson 2014). As diadromous fish occupy a continuum of habitats during their life cycle (freshwater, estuarine and marine), the link between processes occurring in these habitats that influence growth, mortality and survival are critical to understanding their population dynamics (Limburg 2001). In this chapter, I aim to shed light on some of the factors that influence the entire life history of inanga, from hatching through sexual maturity, and relate this to various aspects of their demographics.

To date, considerable research effort on amphidromous fishes has focused on discrete parts of their life cycle. For example, most studies have focused on the spatial ecology of pelagic stages to address implications of dispersal on population structure and connectivity (Waters et al. 2000, Schmidt et al. 2011, Warburton 2015). Patterns of size and age at inward migration, and how these differ over environmental and oceanographic gradients are also widely studied to better understand the broader factors influencing their larval life histories and migrations (Radtke et al. 1988, Bell et al. 1995, Barbee et al. 2011). Likewise, discrete studies on the reproductive dynamics of adults are common. For example, spatial and temporal variation in spawning are studied to ascertain the effects of abiotic conditions on reproduction, as well as to gain some simple species-specific biological information (Keith 2003, Iida et al. 2008, Shen and Tzeng 2008, Barbee et al. 2011, Stevens et al. 2016). Additionally, the reproductive histories of fish are explored to infer the number of spawning events an individual has contributed to, and to better describe their reproductive biology (Ha and Kinzie 1996, Way et al. 1998, Boy et al. 2009b, Teichert et al. 2014, Stevens et al. 2016, Teichert et al. 2016). Relationships between body size and fecundity are also analysed to understand variation in reproductive output (McDowall 1968, Ha and Kinzie 1996, Stevens et al. 2016, Teichert et al. 2016).

Notwithstanding these studies, however, the critical link between pelagic and freshwater development remains poorly understood, and this limits our understanding of the ecology and biology of amphidromous species. Little is known about processes occurring in the early life stages that can shape adult dynamics and demographics. Likewise, relatively little is known about how variation in adult reproductive dynamics and demographics affects the early life histories and dynamics of their offspring. Migration between marine and freshwater environments helps ensure that the benefits of migration outweigh the costs such that diadromous species can maximise their fitness and survival for reproduction (Gross 1987). For diadromous species, an individual's lifetime growth trajectory can be determined early in life

(Vincenzi et al. 2016), and this can affect all aspects of its subsequent life history. For example, early somatic growth underlies variation in vital rates like size and age at sexual maturity, maturation timing, reproductive investment, number of spawning events and egg size (Friedland et al. 1996, Adams and Huntingford 1997, Vollestad et al. 2004, Sloat et al. 2014, Lewis et al. 2015). Furthermore, alternative life history pathways can arise between early and late spawned fish that are related to their growth histories. In white perch (*Morone americana*), for example, earlier hatched larvae that were slower-growing formed the migratory component of the population, whereas faster-growing, later-hatched fish did not migrate but instead remained resident in freshwater (Kerr and Secor 2010). Growth acquired in freshwater and marine habitats of Atlantic salmon (*Salmo salar*) is related to recruitment and abundance because of the effects of growth on their mortality (Ruggerone et al. 2009). Growth is clearly a key factor underlying population dynamics (Ruggerone et al. 2009), that can ultimately affect the numbers of fish that reproduce.

Early life mortality is usually substantial in fish (Bailey and Houde 1989), so information on the characteristics of the adults that survive to maturity can provide valuable insights into the ecology of diadromous fishes (Limburg 2001). One potential technique to link the pelagic and adult life phases of amphidromous species is via analysis of the chronological information contained in otoliths. An individual's life-time growth history can be reconstructed from the daily increment pattern and related to various intrinsic (sex, cohort) and environmental factors (temperature) to understand drivers of growth variation (Pannella 1971). This information can then be related to key demographic factors to interlink growth with the population dynamics of amphidromous fishes.

5.1.1 Relationships between pelagic and adult life in inanga

Inanga (*Galaxias maculatus*) are distributed over 12° latitude in New Zealand (McDowall 1968), with spawning occurring from February through to September (Taylor 2002, Hicks et al. 2013). Substantial spatial and temporal variation in pelagic growth rates have been observed, which is interlinked with their hatching-times (see Chapter 3). During their pelagic phase, age-dependent growth rates are higher for later-hatched fish and somatic growth trajectories can diverge after as little as 30 d for the earlier- and later-hatched fish (see Chapter 3).

Post-larvae typically migrate to freshwaters during the late-winter through spring, but can be observed in lower abundances throughout the year (McDowall et al. 1994). Inward

migration timing is temporally structured such that most autumn-hatched individuals migrate inwards during early spring at significantly older ages than winter-hatched fish that migrate during November (late spring; see Chapter 2 and Rowe and Kelly (2009)). Regional variation in size at inward migration is found in New Zealand, but size is generally more consistent within regions at the river scale (see Chapter 2 and Rowe and Kelly (2009)). Irrespective of hatching and inward migration times, along with their widely different somatic growth rates, post-larvae are of similar sizes at freshwater entry, but for their age, later hatched fish are larger (Chapter 3).

Inanga metamorphose in the lower reaches of rivers, while adult growth and development occurs further upstream for approximately six months (McDowall 1968). There is a dearth of information on basic aspects of the life history of this species such as age at sexual maturity and growth rates during freshwater life. Based on their size frequency distributions, inanga usually live for one year, but some individuals have been shown to survive for up to two years of age (Burnet 1965, McDowall 1968). However, no definitive aging studies have been completed.

The reproductive dynamics of inanga are highly variable and may reflect underlying variation in somatic growth. For example, not all sexually mature adults are large and some mature at half the body size of other fish in the same cohort. Mature females can range from 50 to 125 mm total length (L_T) and males from 50 to 105 mm (Stevens et al. 2016). Stevens et al. (2016) documented iteroparity among New Zealand populations and showed that spawning and subsequent survival is not size-dependent because males and females were iteroparous across a broad size spectrum (Stevens et al. 2016). Size at sexual maturity, body condition and gonad weight tend to decline throughout the spawning season for both sexes (McDowall 1968, Barbee et al. 2011), which might be related to multiple spawning events or that the reproductive dynamics of fish later in the season differ to those that mature earlier. Relationships between body size and fecundity are highly variable, particularly in larger (> 60 mm total length) fish (McDowall 1968). For example, up to six-fold differences in fecundity were found among females that were 80 mm L_T (McDowall 1968). Sexually mature males are typically smaller than females, which suggests there are different life history strategies pertaining to growth and reproduction between sexes (Barbee et al. 2011, Stevens et al. 2016).

5.1.2 Aims and objectives

Systematic differences in pelagic growth histories (see Chapter 3) as well as variable migration dynamics (see Chapter 2) provide an opportunity to explore relationships between pelagic and adult growth. Furthermore, the effects of variable somatic growth on size and age at sexual maturity, as well as reproductive investment can be investigated to provide a link between processes occurring in pelagic and freshwater habitats that affect demographics. In this chapter, the somatic growth trajectories of adult inanga were derived from their otoliths as well as age and hatch dates. I aimed to:

1. Provide the first measurements of age and hatch dates for adult inanga.
2. Characterise the age-dependent somatic growth trajectory of adult inanga and relate this to developmental and life history transitions.
3. Investigate age-dependent somatic growth trajectories of males and females, among hatching times and maturity stages to identify potential underlying factors driving variation in somatic growth.
4. Derive estimates of size and age at maturity and how these differ among hatching times for males and females.
5. Investigate relationships between reproductive investment and somatic growth histories for males and females among hatching times.

5.2 Materials and methods

5.2.1 Sampling

Inanga were sampled from four rivers in Golden Bay (Motupipi, Tukurua, Puremahaia and Yellow Pine; see Chapter 1 for an overview of the study sites) across four months (January-April 2014). Fish were caught using Exotic Gee Minnow traps (3.17 mm wide mesh) baited with Marmite® yeast spread. Traps were set around spawning habitat at the freshwater/estuarine interface upstream to the upper extent of inanga distribution in a river. This was done to ensure that a representative sample of the population was obtained and that sufficient numbers of each sex were captured, as males tend to be more common in lower reaches and females in upper reaches (McDowall 1968). On each sampling occasion, traps were set in the morning and collected in the afternoon.

A total of 70 fish were randomly sampled from the total catch in each river in each month. Fish were transported to the laboratory in 20 L buckets of aerated water until

processing. Individuals were euthanized with 2-phenoxyethanol (1 $mL.L^{-1}$). For each specimen, whole body wet mass (M) and total length (L_T) measurements were taken using a microbalance (±0.01 g) and Vernier calipers (±0.1 mm). Individuals were dissected and sexed based on visual inspection of their gonads. Macroscopic staging of gonads was done to assess reproductive status using the eight stages of gonad criteria identified by Stevens et al. (2016). Stages One through to three were grouped as immature fish, Stages Four to Six were mature and Stages Seven and Eight were spent. The gonads were removed and weighed (M_G) using a microbalance (±0.01 g). A gonado-somatic index (I_G) was calculated for each specimen as a size-standardised measure of reproductive investment:

$$I_G = \frac{M_G}{M} \times 100$$

Otoliths were stored in the intact head in 70% ethanol until extraction.

5.2.2 Otolith preparation and data extraction

A random subset of fish from each month, river and sex (at least ten) were used to estimate age and for back-calculation of hatch dates. A detailed overview of the otolith preparation process is given in Chapter 2. The process of otolith preparation was mostly similar to that of post-larvae except adult otoliths were flipped multiple times and polished on each face to reduce thickness and to visualise the rings. Age was estimated for each fish using counts of daily rings. Hatch dates were estimated by subtracting age at capture from date at capture.

The macrostructure of adult inanga otoliths is considerably variable. Numerous translucent rings were found that likely coincide with life history events like spawning, inward migration and seasonal growth checks (McDowall 1968). This makes ageing using annuli counts difficulty because a ‘true annulus’ cannot be identified and is similar in appearance to translucent ‘false rings’. The zone in the central region of the otolith represents pelagic growth and appears as a translucent zone when viewed in dark field and as a white zone when viewed in differential interference contrast (Fig. 5.1). An opaque or dark zone followed the pelagic zone and often, a translucent ring was found following this first opaque zone. This translucent ring likely represents the juvenile-metamorphosis phase (Fig. 5.1). This ring varied in width and clarity and likely coincides with the time taken to complete metamorphosis (Correia et al. 2003). Examination of the daily increment deposition was therefore a more robust way to age inanga and for growth reconstruction. Increment deposition was usually clear and easily discernible (Fig. 5.2).

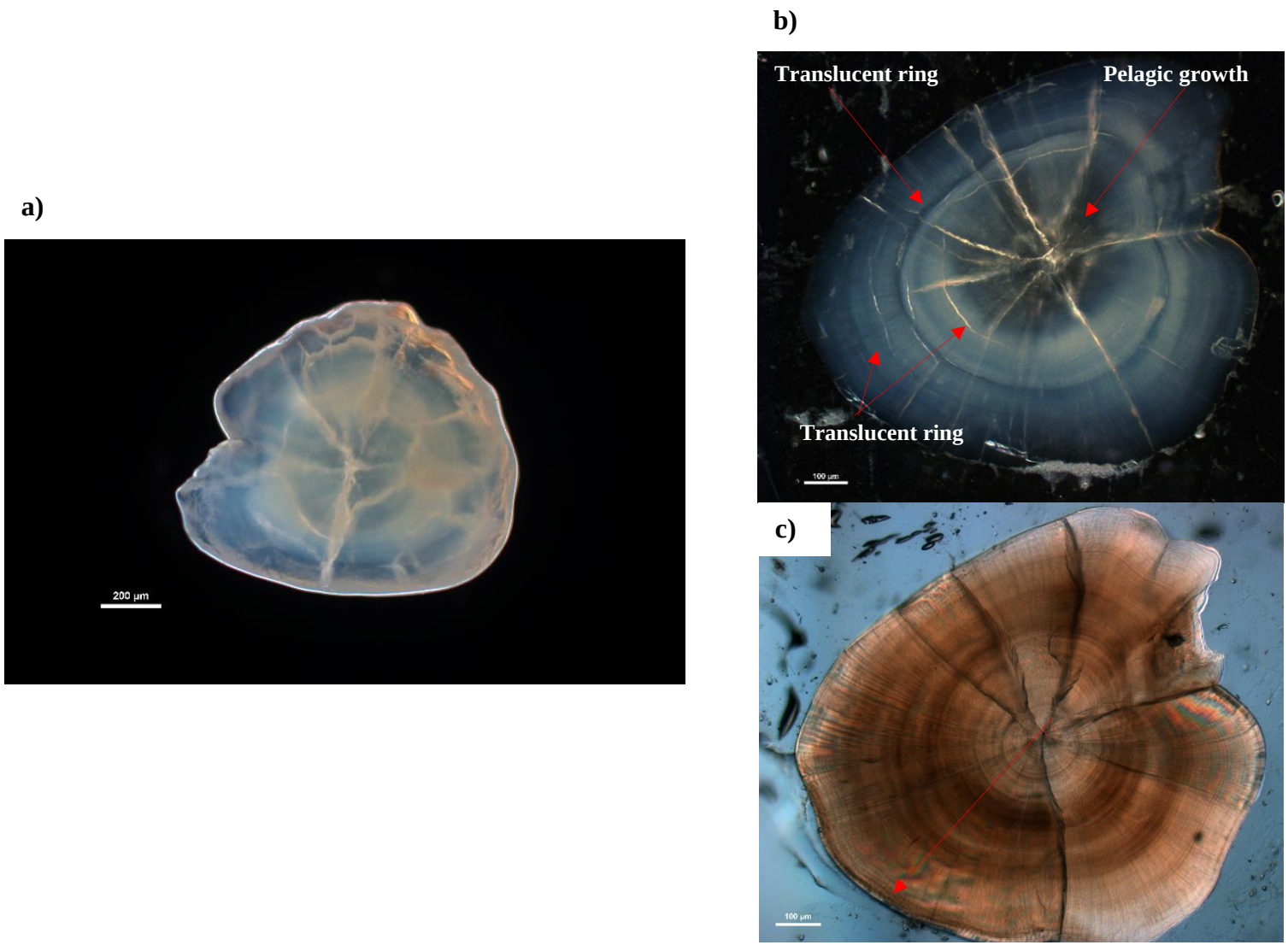


Figure 5.1. Photo-micrograph of **a)** whole adult inanga otolith at 5x magnification. Panel **b)** and **c)** show a polished otolith taken in dark field and differential interference contrast respectively. The macro-structural features of the otolith are shown. In **b)** the red arrow shows the growth zones. In panel **c)** the red arrow shows the axis used for growth reconstruction.

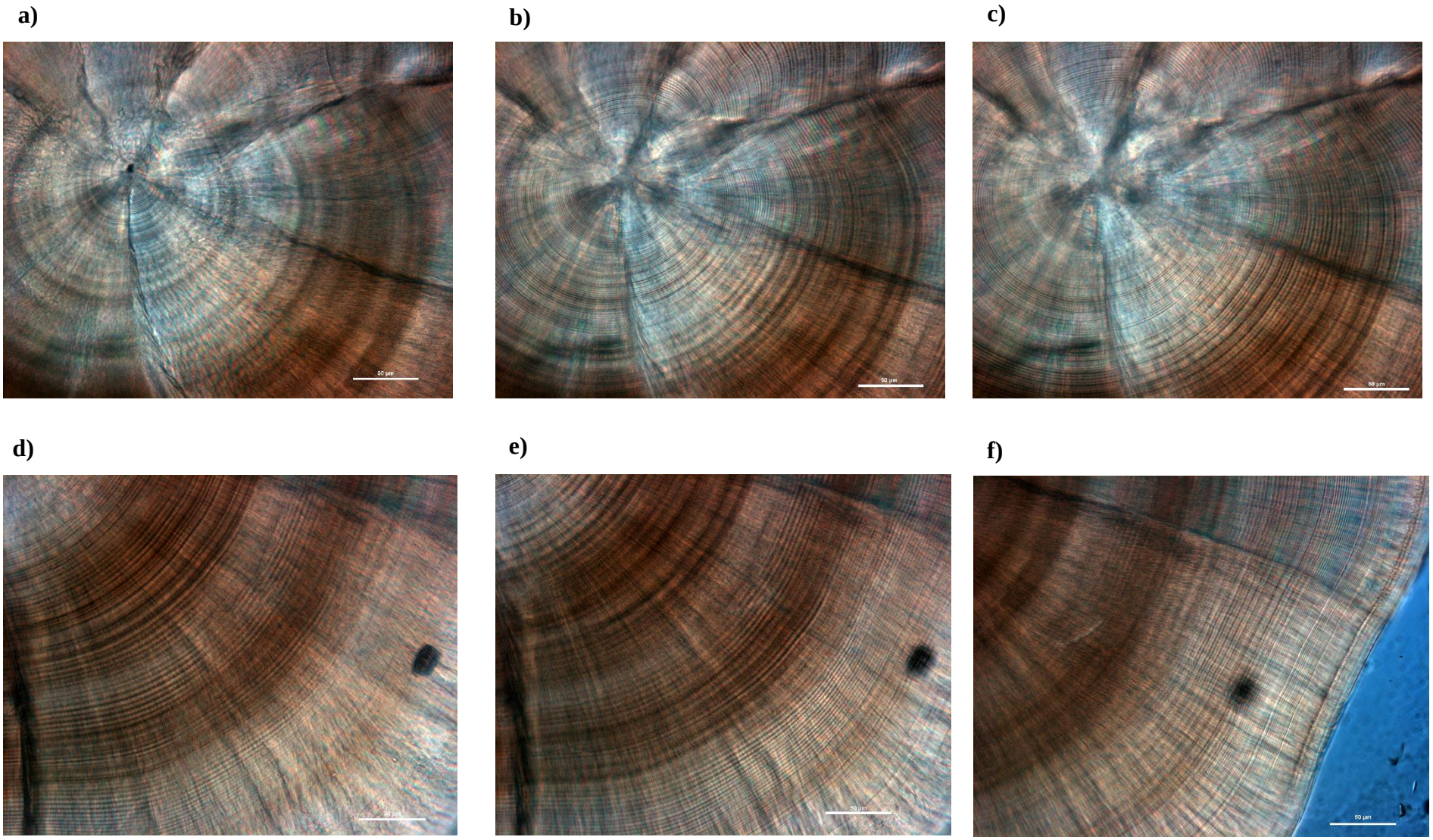


Figure 5.2. Photo-micrograph of adult inanga otoliths taken with a 40x objective. Panels **a-f** show the microstructure and daily increment deposition from the hatch mark (**a)**) to the otolith edge (**f)**).

5.2.2.1 Somatic growth rates

Somatic growth rates (averaged over 10-day intervals, see section 3.2.3 for rational) were estimated from the hatch-mark to the otolith edge. This was done to account for some of the measurement error inherent in otolith increment studies and to reduce noise in the data. This generated a chronology of age-dependent somatic growth rates from pelagic to freshwater stages in 10 d increments.

For this study, somatic growth rates were not converted to retrospective body size-at-age. This is because fish length and otolith length can become uncoupled during metamorphosis (Hale and Swearer 2008), introducing considerable bias and uncertainty in back-calculated size-at-age models (Campana and Neilson 1985). Although the fish-length to otolith-size relationship shows that adult otolith increment measurements are a good approximation of relative changes in fish length (Fig. A.5.1), 28% of the variation in length was unexplained, which may be attributable to uncoupling during metamorphosis, as well as to growth or age effects. Instead, a more conservative approach was taken and the raw somatic-growth data from otolith increments were used to examine changes in growth over the lifetime of inanga.

Somatic growth rates (increment width) and length are highly interrelated in fish; wider increment distances mean higher growth rates and greater increases in fish length (Campana and Neilson 1985). Studies that reconstruct somatic growth histories in diadromous species often use distinctive check marks in the otoliths or scales that clearly demarcate marine growth from freshwater growth (Ruggerone et al. 2009, Marco-Rius et al. 2012, Freshwater et al. 2016). This approach was not feasible for inanga because multiple check marks and translucent rings that are evident in the otolith have yet to be formally validated (see Chapter 2). While analysis of the somatic growth trajectory does not directly relate pelagic to freshwater growth, the shape of the growth trajectory, coupled with prior knowledge of the post-larval growth trajectory, can be used to demarcate pelagic growth from freshwater growth. As a result, growth rates during the pelagic and freshwater phases can be directly compared at a given age.

5.2.2.2 Assigning growth increments to 'environmental proxies'

Because of the temporal nature of otolith increment deposition, each growth increment can be assigned a date at formation. Although inanga were sampled across four months (January – April), they all shared at least a portion of their growth in the pelagic and freshwater habitats

during the same time of the year. The extent of this overlap depends on their hatch date and the timing of freshwater entry (see Chapter 2).

Assigning increments to a date at formation derives a temporally resolved 'environmental proxy' for growth (Morrongiello and Thresher 2015), and is useful when the specific environmental conditions experienced by an individual are unknown. Using this approach, the likely environmental history can be inferred because higher or lower growth during particular times of the year can be coupled with what is known about inter-annual and seasonal variation in the environment (Folkvord et al. 2016). This approach also incorporates differences in the length of the growing season and is a proxy for day length (Hinrichsen et al. 2010).

To derive an environmental proxy, the calendar date at formation for the first increment is an individual's hatch date (back-calculated) plus 1 d. The calendar date when the tenth increment was formed is an individual's hatch date plus 10 d. I then calculated the average date at formation of increments over 10 d intervals to assign each 10-d interval a mean date (day of the year) at formation.

5.3 Data analysis

The analysis is split into three sections. First, a subsample of fish across months was used for ageing, back-calculated hatch dates and growth modelling. Next, size and age at sexual maturity, as well as reproductive investment were investigated across hatching times. Finally, the characteristics of all fish sampled in the four studied rivers (total length, gonad development and reproductive investment) across months, between sexes and maturity stages were investigated and these results are stored in Appendix 5 A.5.2.

5.3.1 Ageing and hatch dates

A second set of analyses involved Kolmogorov-Smirnov tests to test for differences in the hatch date distribution between males and females. Pearson's correlations were used to explore relationships between size and age for males and females.

Linear mixed effect models (LMEMs) were initially used to test for differences in size and age at sexual maturity among hatching times and between sexes. However, these analyses showed that river was not a significant source of variation in size or age at sexual maturity, so a simpler ANOVA was used.

5.3.2 Modelling growth using generalised additive mixed models

A third set of analyses involved generalised additive mixed models (GAMMs) to analyse somatic growth trajectories (otolith increment widths). GAMMs were used because exploratory analysis showed that age-dependent growth trajectories were non-linear. GAMMs do not specify a functional relationship between predictors and the response variable, and they can fit linear and non-linear relationships for the predictor variables of interest in one statistical framework (Wood 2006). Additionally, measurements of otolith increment widths are inherently auto-correlated spatially and temporally unbalanced and non-independent (Weisberg et al. 2010). GAMMs can account for some of these issues by specifying the appropriate random effects structure, as well as modelling auto-correlation in the residuals (Wood 2006). GAMMs have been used to examine age-dependent somatic growth rates in small, short-lived fish (Baumann et al. 2006, Hinrichsen et al. 2010, Schismenou et al. 2016).

To model somatic growth trajectories, multiple GAMMs were fitted using a suite of predictor variables, categorised as fixed intrinsic (ontogenetic and additional), extrinsic (spatial and temporal) and random effects (Table 5.1). $Age_{increment}$ models age-dependent changes in somatic growth at each ten-day interval over an individual's lifetime. Hatch-season is a 'catch all' term that represents differences in growth rates associated with timing of reproduction (autumn/winter/spring), varying pelagic growth rates, and timing of entry into freshwater (see Chapters 2 and 3). The factor sex was used to test for differences in somatic growth rates between males and females. The factor maturity was used to examine differences in somatic growth rates among fish of different sexual developmental stages (immature/mature/spent). Julian day tests for differences in growth over the length of the growing season. Individual fish were modelled as random effects to account for repeated measures inherent in otolith increment width studies and random variation due to differences between individuals (Weisberg et al. 2010). Fish were nested within rivers to account for variation in growth among rivers. Random slopes were investigated to test for different age-dependent growth trajectories among individual fish.

To model non-linear trends in somatic growth rates, smoothers were fitted to each continuous predictor variable (Table 5.1) using cubic regression splines These smoothers use shrinkage terms so that the optimum number of 'knots' capturing non-linear relationships can be estimated using generalized cross-validation (Wood 2006).

Table 5.1. Predictor variables used to model otolith increment width (μm) (as a proxy for somatic growth) using generalised additive mixed models.

	Fixed effects		Random effects
Intrinsic		$Age_{increment}$ (days, continuous) Sex (male/female) Hatch-season (autumn/winter/spring, factor) Maturity (immature/mature/spent, factor)	Fish_ID (intercept)
			–
Extrinsic	Spatial	–	River (factor)
	Temporal	Days (continuous)	–

GAMM outputs include the term effective degrees of freedom (EDF) for each continuous smoother. EDFs range from zero to infinity, with higher values implying greater non-linearity in the relationship with a continuous predictor (Wood 2006). Shrinkage reduces the degree of smoothing for each continuous predictor so that predictors with a non-linear effect are shrunk to an EDF of one or less. To visualise the effects of continuous smoothers, partial effects plots are produced. These plots show the non-linear effect of the smoother while holding all other variables in the model at a constant. If an interaction is fitted for a continuous smoother, each factor level of the predictor is mean-centred. The smoother therefore does not include systematic differences in mean values for the factor level on the response so that comparisons can be made on the same scale (Wood 2006).

GAMMs were fitted in a three-step process. Intrinsic ontogenetic factors were fitted first for each model because they are the primary factors that influence fish growth (Morrongiello and Thresher 2015). Next, the optimal random effect structure was specified using restricted estimates of maximum likelihood (REML) by increasing the complexity of the random effects (intercepts, nesting and slopes). Once complete, model assumptions (normality of the residuals, residuals against linear predictor, response against fitted values and deviance residuals against theoretical quantiles) were examined using the gam.check function in R. Residual auto-correlation structures were incorporated when necessary by using auto-regressive correlation structures (corAR1). At each stage of the process, competing random effects and auto-correlation structures were ranked and the optimal model was identified using Akaike's information criterion (AIC), where the lowest AIC is the optimal model (Zuur et al. 2009). Where competing model structures were identified, the model with the lowest numbers of variables was chosen.

The base model was used to assess the significance of the relevant predictors depending on the model of interest. Increasingly more complex models were compared using Likelihood ratio (LR) tests against the base model to decide on the significance of terms (Zuur et al. 2009).

The EDF scores for smoothers of continuous predictors and their relevant interactions were inspected. If the EDF was less than 1, the model was refitted with a linear term for that predictor.

Optimum models were refitted using REML to obtain unbiased parameter estimates (Zuur et al. 2009). Approximate tests of significance were done on the optimum model using Wald's tests (anova.gam) on the smooth and parametric terms. Otolith increment widths were assumed to follow a Gaussian distribution with mean and variance equal to μ and σ^2. All statistical analyses were performed in R 3.2.2 (R Core Development Team 2015). GAMMs were fitted using the mgcv package.

5.4 Results

In total, 1,034 fish were sampled from the four rivers across four months (January-April). A detailed breakdown of fish numbers sampled in each river across months is given in the supplementary analysis section (Fig. A.5.2).

5.4.1 Ageing and hatch dates

A random subsample of fish was chosen from the total pool of fish sampled in each month across all rivers. During the otolith preparation process, there were some difficulties obtaining a clear growth sequence that spanned pelagic to adult growth. Otoliths of larger fish (> 100mm L_T) were more difficult to age because of their increased thickness preventing sufficient penetration of light to visualise daily rings. Although more than 200 adult otoliths were polished, the final dataset of adult growth chronologies varied between sexes and months. This resulted in 119 adults with their growth increments measured. Of these, 67 were female, 38 male and 14 had undetermined sex. Overall, 53 fish were sexually immature, 61 mature and 5 spent (Table 5.2).

Table 5.2. Numbers of adult inanga that were aged between sexes, maturity stages and among months.

		Capture month			
Sex	**Maturity**	**January**	**February**	**March**	**April**
Female	Immature	17	10	–	7
	Mature	5	11	7	6
	Spent	–	1	–	3
Male	Immature	3	2	–	–
	Mature	7	10	4	11
	Spent	–	–	1	–
Undetermined	Immature	13	1	–	–
Total		45	35	12	27

Fish were grouped into early- (January and February caught) and late- (March and April caught) classes for further analysis because of the highly unbalanced dataset. Aged males and females spanned a range of developmental stages (Fig. 5.3). All immature fish with undetermined sex were caught early in the season, with most immature males and females also found during this period. Approximately equal numbers of mature males and females were aged during the early- and late-sampling period (Fig. 5.3).

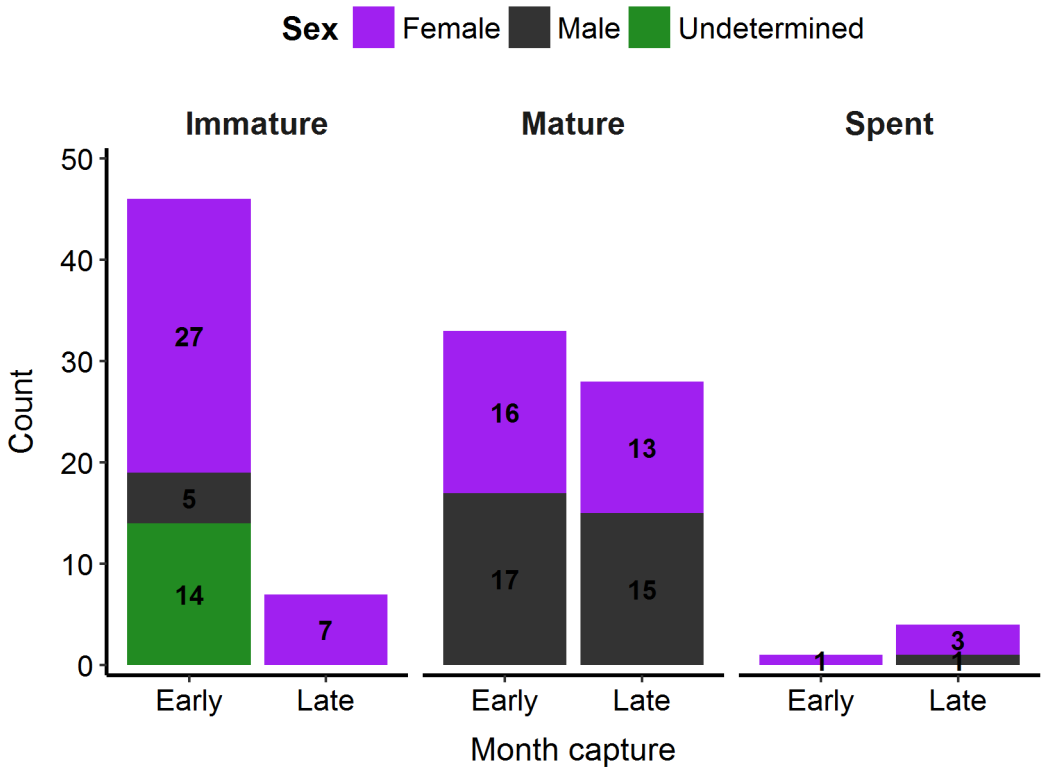


Figure 5.3. Stacked bar chart showing the numbers of aged adults among month at capture (early or late), maturity stages and sex.

5.4.1.1 Relationships between size and age

Overall, aged females were between 149-344 d old (mean = 249, SD = 45.4) and on average 74.5 mm L_T (SD = 11.55, range = 49.7 – 95.1 mm). Males were between 194-350 d old (mean = 256, SD = 41.8,) and average L_T was 74.2 mm (SD = 5.7, range = 63.4 – 88.5 mm). Immature indeterminate fish were 56.1 mm L_T (SD = 2.63, range = 49.9 – 61.3 mm) and 215.14 d old (SD = 28.6). Correlations between L_T and age for males ($r = 0.54$, $p < 0.001$) and females ($r = 0.72$, $p < 0.001$) were positive, showing that larger fish were mostly older, although there was greater variation in this relationship for males (Fig. 5.4).

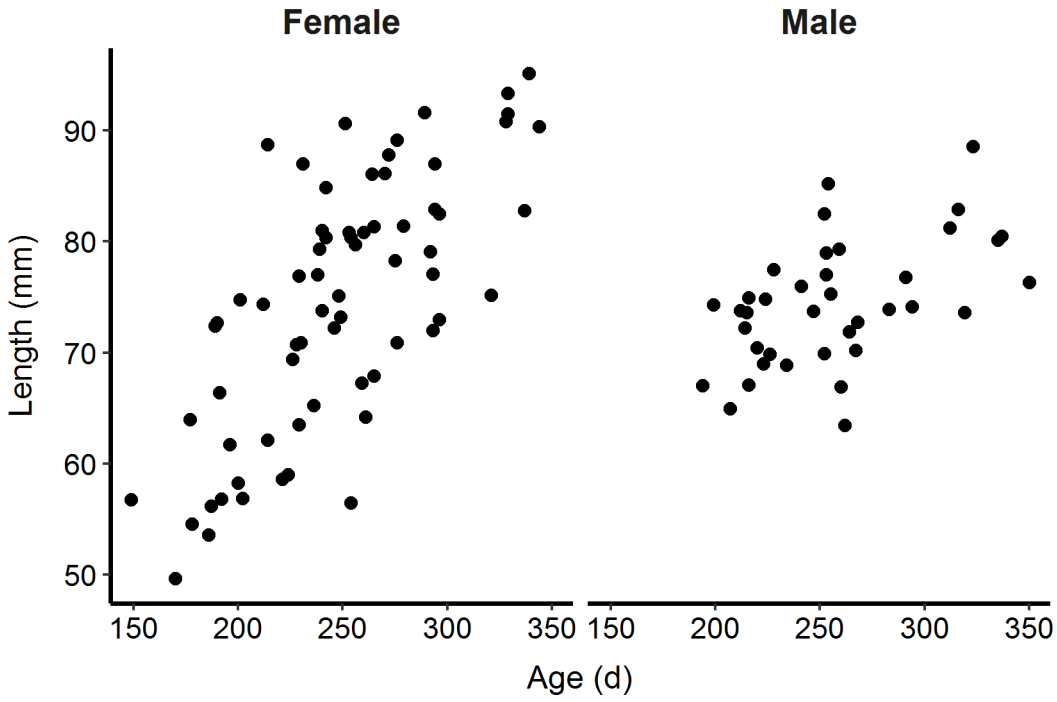


Figure 5.4. Relationship between age (d) and total length (mm) of adult inanga for each sex.

5.4.1.2 Hatch dates

From the pool of aged fish, the back-calculated hatch dates ranged from mid-February 2013 to September 2013. The distribution of hatch dates did not differ among sexes (KS test, $p > 0.1$, $D = 0.22$), showing that males and females hatched throughout the year. Immature indeterminate fish hatched from April to July 2013. Too few fish were identified in each hatch-month category within and between sexes, and months, to allow for statistical comparison (Fig. A.5.3). Therefore, hatch-months were grouped into hatch-seasons: summer (February), autumn (March - May), winter (June- August) and spring (September) to increase sample sizes.

Most of the immature males and females during the early season were winter-hatched (Fig. 5.5). Of all early-mature females, 62% were autumn-hatched, 6% spring-hatched and 31%

winter-hatched (Fig. 5.5). Approximately equal proportions of early-season mature males were autumn-hatched and winter-hatched (Fig. 5.5). Of all mature females during the late-season, 92% were winter-hatched. For late-season mature males, 62% were winter-hatched and 32% spring-hatched. Late-season immature females were comprised of equal proportions of autumn-, winter-, and spring-hatched fish (Fig. 5.5), although there were fewer than three fish in each hatch-season category. No late-season immature males were available in the aged dataset. Few males were immature during the late season from all fish collected (see Fig. 5.3).

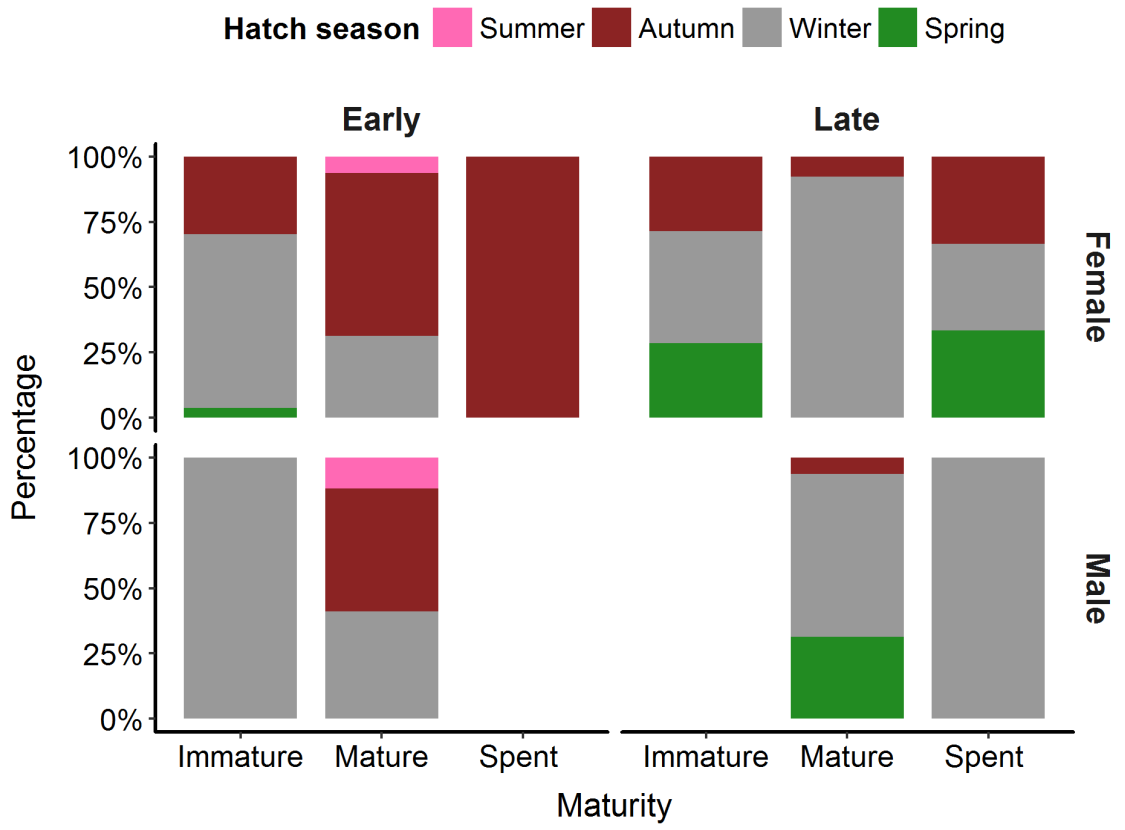


Figure 5.5. The proportion (expressed as a percentage) of male and female fish that were hatched across seasons that were immature, mature or spent during the early- or middle-season.

5.4.1.3 Size and age at maturity among hatch-seasons

Size and age of males and females across hatch-seasons and capture times could not be compared because of the highly-unbalanced sample sizes. Therefore, early- and late-captured adults were pooled to increase sample size for analysis of size and age at maturity (Fig. 5.6). Spring and summer-hatched fish could not be analysed because of their small sample sizes.

Overall, mean age of mature females was 264.5 d and L_T was 81.21 mm. Mean age of mature males was 262.8 d and L_T was 74.86 mm. Size-age correlations showed that larger

mature autumn-hatched males ($r = 0.79$, $p < 0.01$) and females ($r = 0.85$, $p < 0.001$) were older (Fig. A.5.3). No such relationship was observed for either sex for mature winter-hatched fish (males: $r = 0.48$, $p > 0.05$; females: $r = 0.27$, $p > 0.1$, Fig. A.5.4).

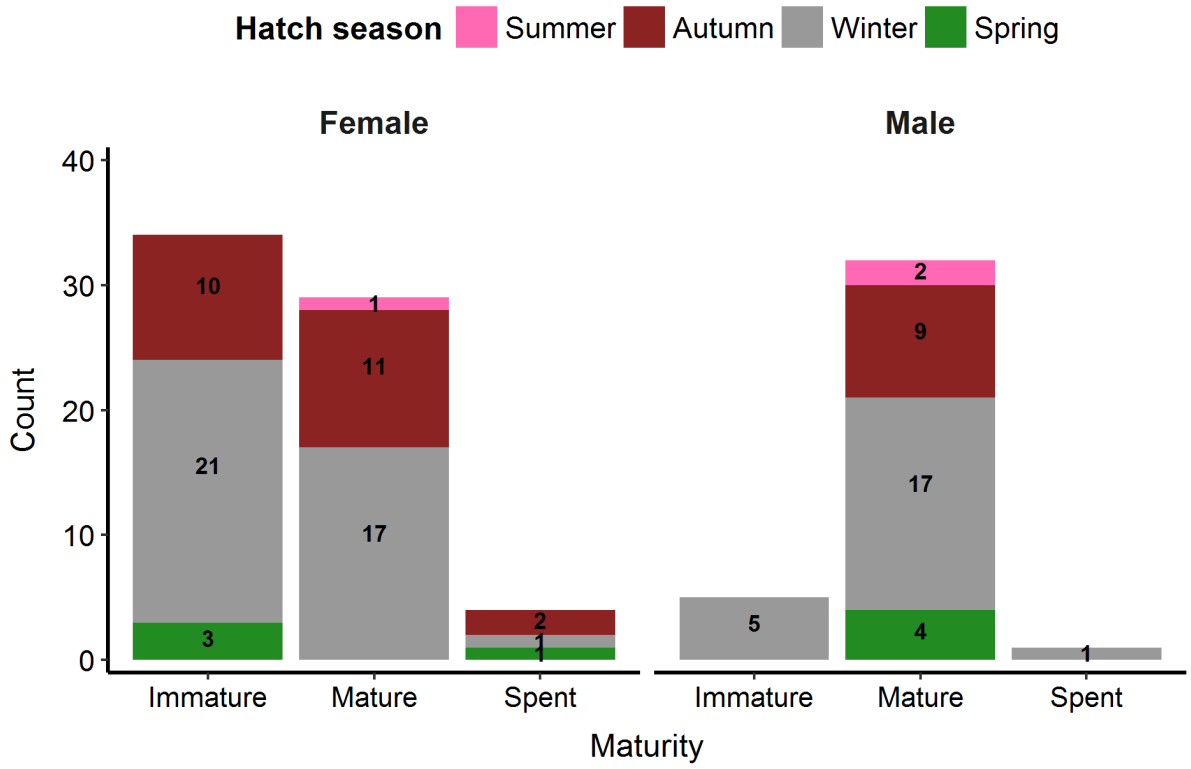
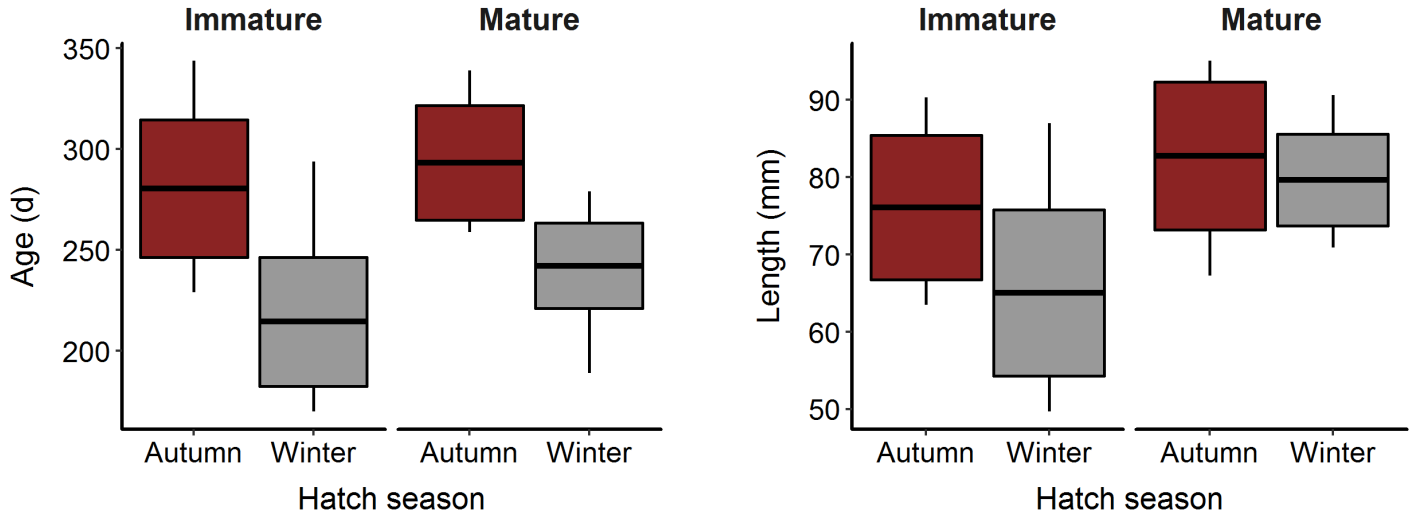


Figure 5.6. Numbers of males and females in each hatch-season among maturity stages between sexes.

There was substantial variation in age and size at maturity for males and females within and among hatch-seasons (Fig. 5.7). Mature winter-hatched females were significantly younger compared to autumn-hatched females ($F_{1,26} = 29.70$, $p < 0.001$, $\beta = -51.13$), but no differences in size of mature fish were found ($F_{1,26} = 1.12$, $p > 0.1$). Mature winter-hatched males were younger than mature autumn-hatched males ($F_{1,24} = 14.6$, $p < 0.001$, $\beta = -45.34$), but not significantly smaller ($F_{1,24} = 0.01$, $p > 0.5$, Fig. 5.7). Immature winter-hatched females were significantly younger than immature autumn-hatched females ($F_{1, 29} = 27.8$, $p < 0.001$), and significantly smaller ($F_{1,29} = 7.74$, $p < 0.01$, Fig. 5.7).

A) Females



B) Males

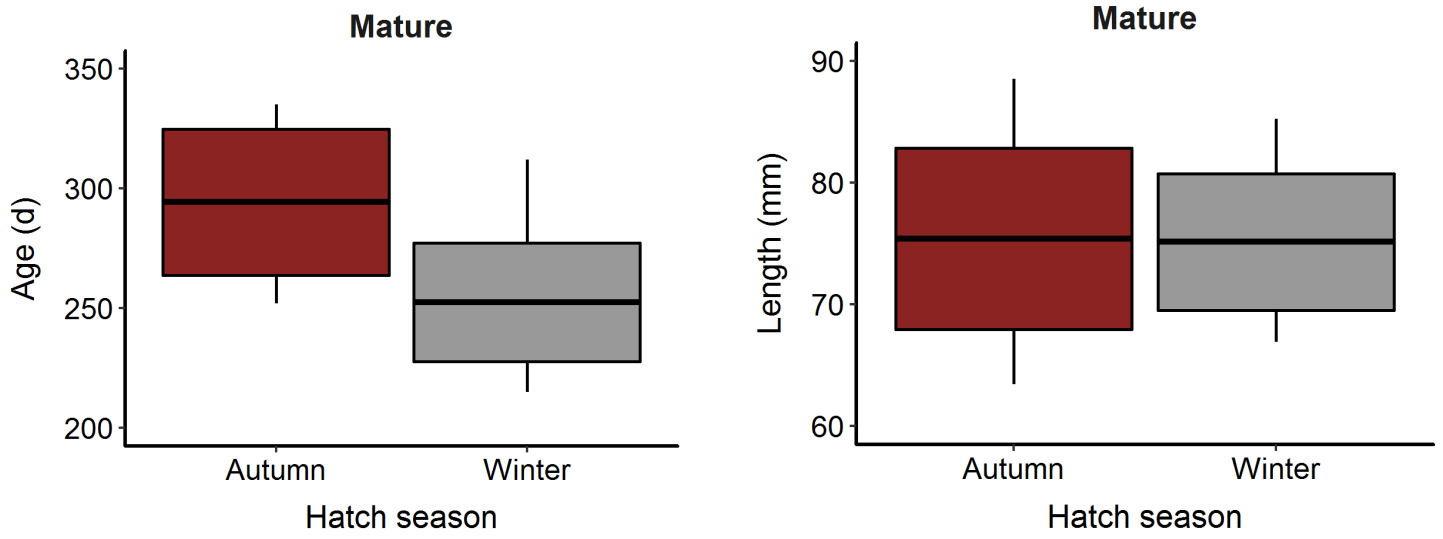


Figure 5.7. Mean age and total length of immature and sexually mature adults among hatch-seasons of **a)** females and **b)** males. Lines are the maximum and minimum values observed. The height of the boxes is the within-sample standard deviation.

5.4.1.4 Reproductive investment

5.4.1.4.1 Females

Relationships between gonad weight (M_G) and body size (L_T) showed there was no relationship for mature autumn-hatched females ($r = 0.47$, $p > 0.1$) but there was for winter-hatched females ($r = 0.53$, $p < 0.05$, Fig. A.5.5). No relationship was found between M_G and age for either mature autumn-hatched ($r = 0.46$, $p > 0.05$) or winter-hatched females ($r = 0.46$, $p > 0.1$, Fig. A.5.5).

5.4.1.4.2 Males

For mature autumn-hatched males, M_G was not correlated with L_T ($r = 0.59$, $p > 0.05$) or age ($r = 0.18$, $p > 0.5$, Fig. A.5.5). Mature winter-hatched males showed a strong positive relationship between M_G and L_T ($r = 0.68$, $p < 0.001$), but relationships between age and M_G were not significant ($r = 0.49$, $p > 0.05$, Fig. A.5.5).

The two-way interaction term testing for differences in the gonadosomatic index between sex and hatch-season for mature adults was not significant (ANOVA $F_{1,50} = 1.83$, $p = 0.18$, Fig. 5.8). Overall, no significant differences were found between hatch-seasons (ANOVA $F_{1,50} = 0.07$, $p = 0.78$) or sex (ANOVA $F_{1,50} = 0.37$, $p = 0.55$).

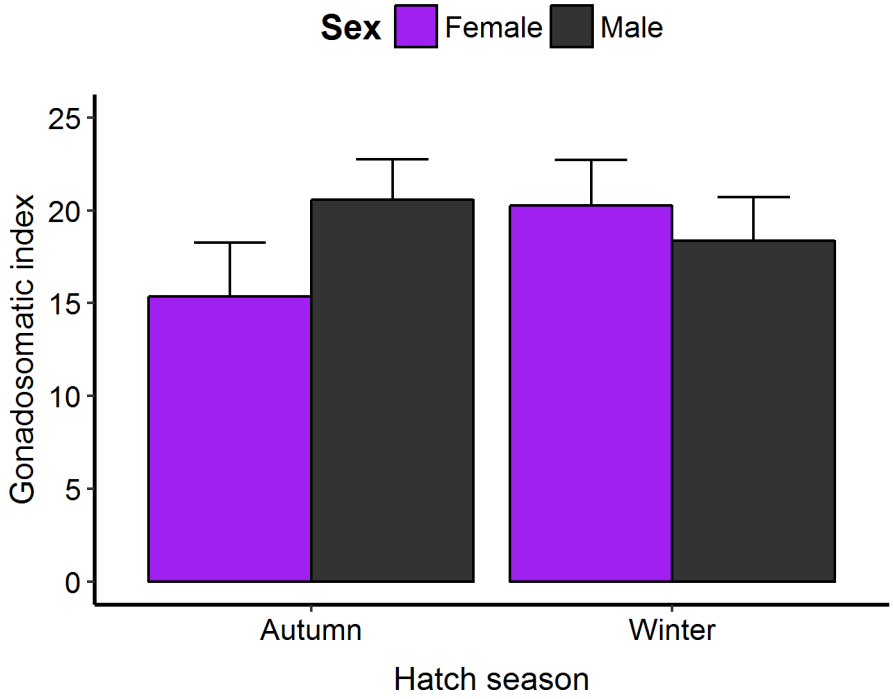


Figure 5.8. Mean gonadosomatic index of mature autumn- and winter-hatched fish between sexes. Error bars are + 1SE of the mean.

5.4.2 Growth modelling

5.4.2.1 Developmental trajectory

Otolith increment widths (a proxy for growth rates) ranged from 0.87 to 4.87 µm. The age-dependent growth trajectory of adult inanga showed a dome-shaped pattern (Fig. 5.9). Growth increased steadily with age during the pelagic phase with an inflection point around 30 d. Growth increased more rapidly between 40 and 80 d and a second growth phase followed whereby growth increased rapidly until a plateau was reached at 120 d (Fig. 5.9). This plateau demarcates the transition from the post-larval 'whitebait' stage to the juvenile stage (metamorphosis) and lasted for approximately 50 d. Growth then decreased rapidly from 170 d, coinciding with the transition from juvenile to adults, and further decreased at 250 d through to sexual maturity at approximately 263 d (Fig. 5.9). In total, 25% of the variation in growth was explained by age-dependent changes in somatic growth (EDF = 7.42, df = 9, F = 125.0, $p < 0.001$).

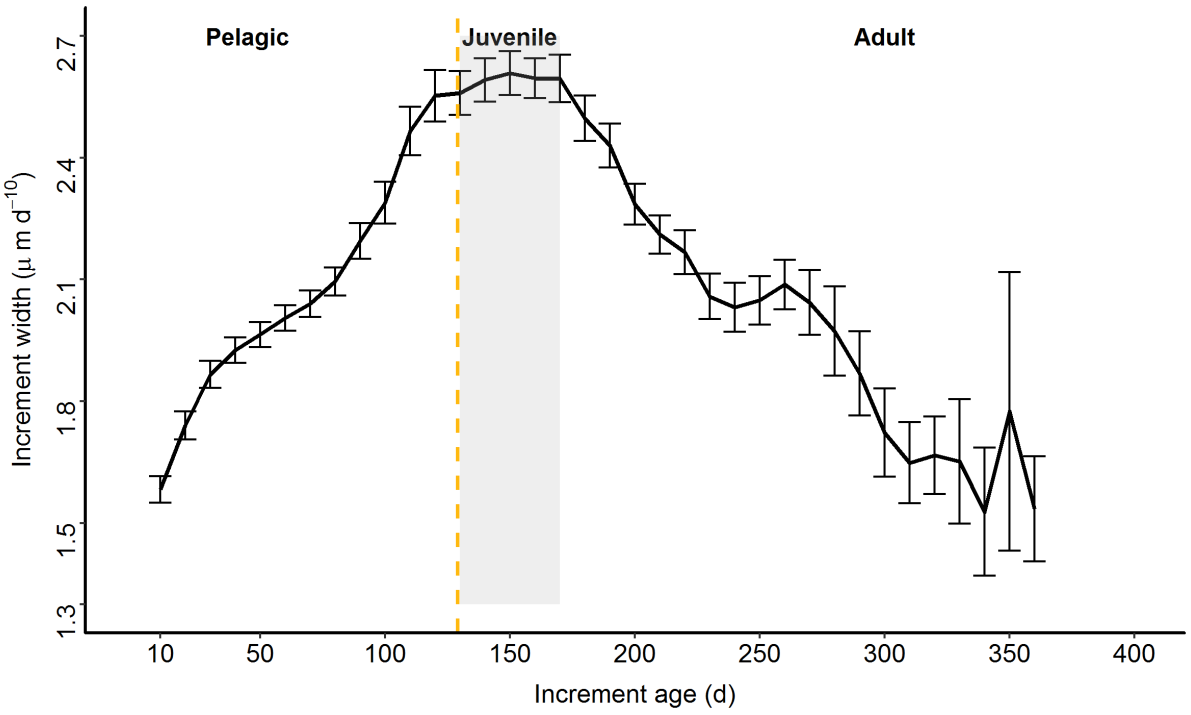


Figure 5.9. Mean age-dependent otolith increment widths as a proxy for somatic-growth (± SE) of adult inanga at 10 d intervals. The orange dashed line is the mean age at inward migration of post-larvae in Golden Bay (see Chapter 2). The grey shaded panel demarcates the juvenile/metamorphosis stage from the larval pelagic and adult freshwater stages.

5.4.2.2 Random effects and auto-correlation

Mean daily growth rates varied substantially among individuals (Fish_ID random intercept, σ = 0.25). Furthermore, there was evidence that mean daily growth differed among fish nested

within rivers (LR = 6.64, df = 5, $p < 0.01$). The contribution of river-specific differences (random intercept river) was considerably less ($\sigma = 0.09$) than inter-individual differences meaning that most of the variation in growth was due to inherent differences among individual fish.

Once the effect of $Age_{increment}$ was incorporated, as well as the random effects for fish nested within rivers, residual autocorrelation was inspected. There was significant autocorrelation between successive increment measurements showing a distinctive pattern (Fig. 5.10). Growth during the first 50 d of life in the pelagic environment was significantly and positively auto-correlated. A change in the direction of autocorrelation then occurred and from 60 – 180 d growth was negatively auto-correlated (Fig. 5.10). The inclusion of a CorAR1 correlation structure within individual fish gave a significant improvement in model fit (LR = 18.88, df = 6, $p < 0.001$) and sufficiently removed autocorrelation. The optimum base model with a fixed effect for $Age_{increment,}$ random effect for fish (river) and autocorrelation was used in all subsequent models.

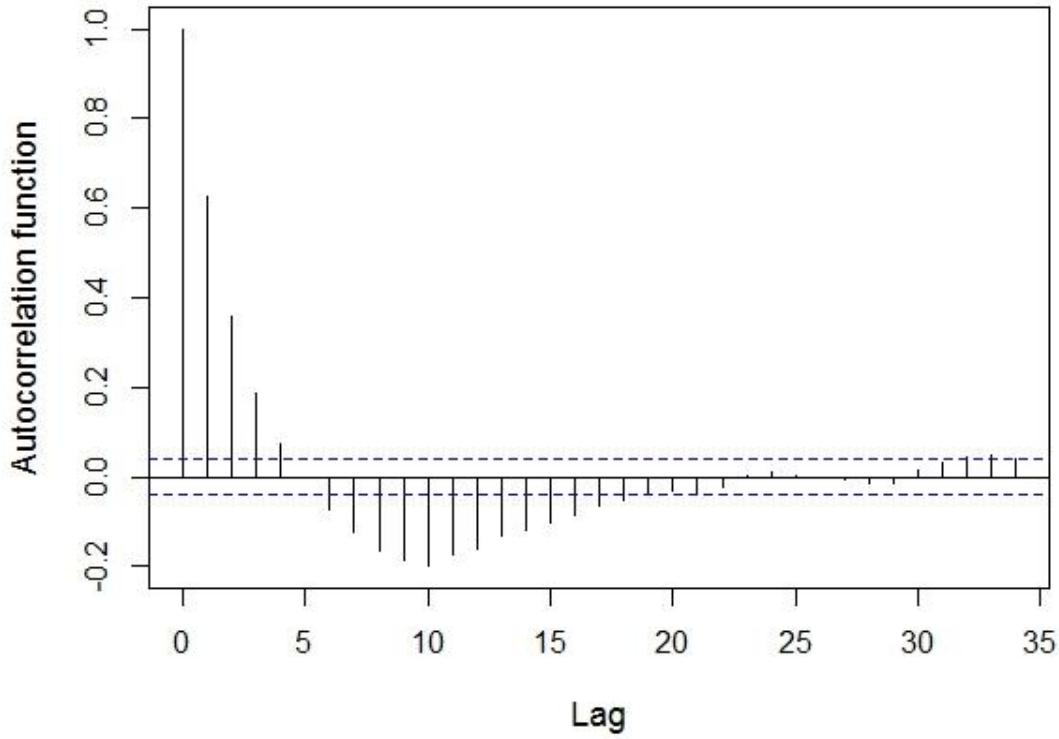


Figure 5.10. Residual autocorrelation from the GAMM among successive increment widths within fish. Each line represents the 10 d intervals in growth. The blue dashed lines show the significance of the autocorrelation at $\alpha = 0.01$.

5.4.2.3 Within hatch-seasons

The effect of day (proxy for temporal variation in abiotic conditions) on growth was investigated for each hatch-season's model. There was no evidence of a significant effect of

day on the growth of autumn-hatched fish once the intrinsic ontogenetic effects of growth ($Age_{increment}$) and serial auto-correlation among successive growth increments within each individual were accounted for (LR = 1.26, df = 6, p > 0.1). The addition of a temporal growth trend for spring-hatched fish did not converge and is probably related to the low numbers of spring-hatched fish (n = 8). For winter-hatched fish, there was a significant effect of day on growth (LR = 5.0, df = 6, p < 0.05). Growth increased from May (150 d) to November (300 d), then declined from December (320 d) until April (470 d) the subsequent year (Fig. 5.11).

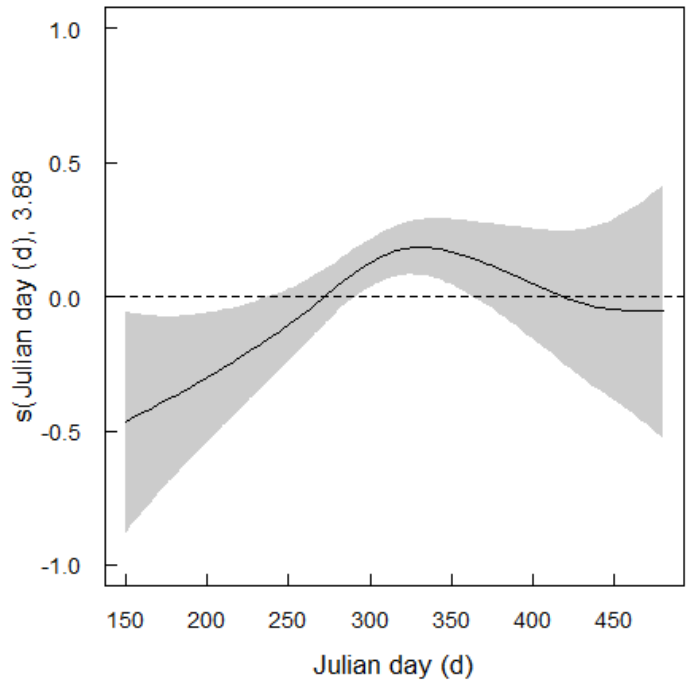


Figure 5.11. Partial effects plots from the GAMM for winter-hatched adults showing the effect of day on growth. The estimated degrees of freedom (EDF) are shown on the y-axis. The dashed line is the model intercept and the grey shaded lines are the 95% confidence intervals of the predicted smoothers.

5.4.2.4 Among hatch-seasons

Significantly different trends in age-dependent somatic growth rates were found for autumn-hatched (F = 9.81, p < 0.001), winter-hatched (F = 37.69, p < 0.001) and spring-hatched (F = 18.56, p < 0.001) fish. These differences among hatch-seasons accounted for 35% of the variation in adult growth. Plots of the raw data are shown in Fig. A.5.6. Age-dependent growth showed a consistent dome-shaped trajectory across hatch-seasons, but growth varied in magnitude with age. For autumn-hatched fish, growth during the pelagic phase was slower and showed the shallowest increase with age by comparison to spring-hatched fish, which grew considerably faster during their pelagic larval phase (Fig. 5.12). Winter-hatched fish were also characterised by steeper pelagic larval growth rates relative to autumn-hatched fish, but the rate

of increase was more gradual than that of spring-hatched fish (Fig. 5.12). The timing and duration of maximum increment widths varied among hatch-seasons. Maximum growth rate was attained at progressively younger ages from autumn-hatched to spring-hatched fish. Autumn-hatched fish had maximum growth at approximately 170 d, winter-hatched at 150 d and spring-hatched at 130 d (Fig. 5.12). The duration of maximum growth was the longest in autumn-hatched fish by comparison to winter-hatched and spring-hatched fish which showed an increasingly distinctive inflection point following maximum growth (Fig. 5.12).

During the freshwater growth phase, winter-hatched fish showed a steeper decline in growth relative to autumn-hatched fish (Fig. 5.12). At 200 d, autumn-hatched and winter-hatched fish had similar growth rates, but older (> 200 d) autumn-hatched fish had higher growth rates than winter-hatched fish (Fig. 5.12). Spring-hatched fish showed the steepest decline in growth during the freshwater phase but remained faster growing than autumn- and winter-hatched fish at ages 150 – 230 d (Fig. 5.12).

5.4.2.5 Within hatch-season, between maturity stages and sex

Mature autumn-hatched males and females did not have different growth trends (LR = 0.00, df = 7, $p > 1.0$), and there was no evidence that mean growth differed between mature males and females (LR = 2.04, df = 7, $p > 0.1$). Similarly, mature winter-hatched males and females did not have different growth trends (LR = 0.00, df = 7, $p > 1.0$), and there was no evidence that mean growth differed (LR = 2.66, df = 7, $p > 0.1$). The growth trajectories of immature autumn-hatched fish could not be compared between sexes because no immature autumn-hatched males were aged. Finally, immature winter-hatched fish did not have different age-dependent growth rates (LR = 0.00, df = 7, $p > 0.1$), and there was no evidence that mean growth differed (LR = 1.91, df = 7, $p > 0.1$).

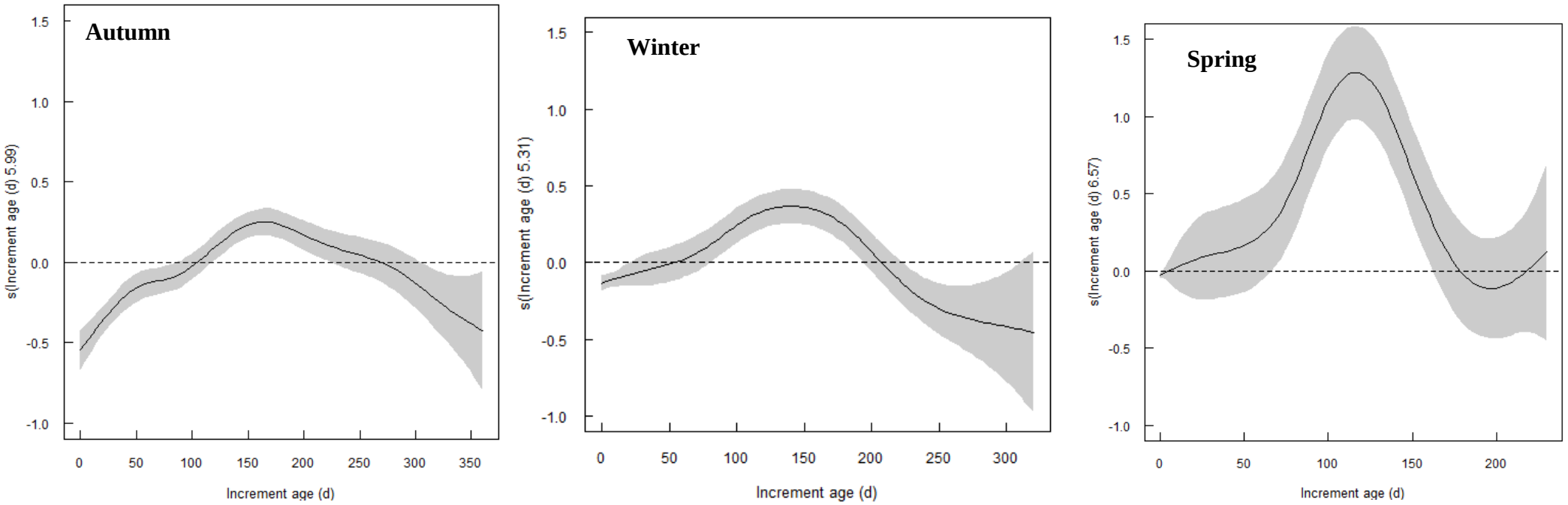


Figure 5.12. Partial effects plots from the GAMMs for fitted smoothers of age-dependent growth rates among hatch-seasons. The estimated degrees of freedom (EDF) are shown on the y-axis. The dashed line is the model intercept and the grey shaded lines are the 95% confidence intervals of the predicted smoothers. Note the varying scales on the x-axes.

5.4.2.6 Within hatch-season between males and females

Mature and immature fish were pooled for each sex and hatch-season. The age-dependent growth trajectories of males and females within each hatch-season were then examined. Age-dependent somatic growth rates of autumn-hatched males and autumn-hatched females overlapped and did not show significantly different trends ($df = 9$, $F = 0.0$, $p > 0.1$, Fig. 5.13). Overall, autumn-hatched males had, on average, lower daily growth rates than females ($\beta = 2.03$) but this effect was not significant ($\beta = -0.06$, $SE = 0.11$, $p > 0.5$). Winter-hatched males and females did not show significantly different age-related growth trends ($df = 9$, $F = 0.0$, $p > 0.5$, Fig. 5.13) and no differences in mean daily growth were found between sexes (female $\beta = 2.15$, male $\beta = -0.28$, $SE = 0.09$, $p > 0.5$). Too few spring-hatched-fish were identified to fit a GAMM for males ($n = 5$) and females ($n = 3$).

Males and females in each hatch-season were pooled and differences among all males ($n = 38$) and females ($n = 67$) were tested. No significant differences were found ($p > 0.5$, Fig. A.5.7). A model with a linear term was fitted instead to test for overall differences in mean growth rate between sexes. Males had lower growth rates than females (intercept = 2.10) but this was not significant ($\beta = -0.04$, $SE = 0.07$, $p > 0.5$).

5.4.2.7 Between sexes and maturity stages

Across hatch-seasons, sexually mature females showed significantly different trends in age-dependent growth ($LR = 18.6$, $df = 7$, $p < 0.001$, Table A.5.1). Mature autumn-hatched females had higher growth rates for their age during their freshwater growth period compared to mature winter-hatched females (Fig. A.5.8). The EDF scores for mature winter-hatched females reflect the steeper declines in growth during their freshwater phase (Fig. A.5.8). Significant differences in age-dependent growth were also found among mature autumn-hatched, winter-hatched and spring-hatched males ($LR = 12.0$, $df = 9$, $p < 0.01$). These differences were mostly driven by the contrasting growth patterns between autumn-hatched and spring-hatched males. For most of their life, spring-hatched males had greater growth compared to autumn-hatched males, although there was considerable variation in this relationship (Fig. A.5.9). Mature winter-hatched males had steeper declines in freshwater growth compared to autumn-hatched, but their age-dependent growth rates were similar from 200 d onwards (Fig. A.5.9). Overall, 40% and 36% of the variation in growth of mature females and males respectively was explained by differences between hatch-seasons.

Between sexes

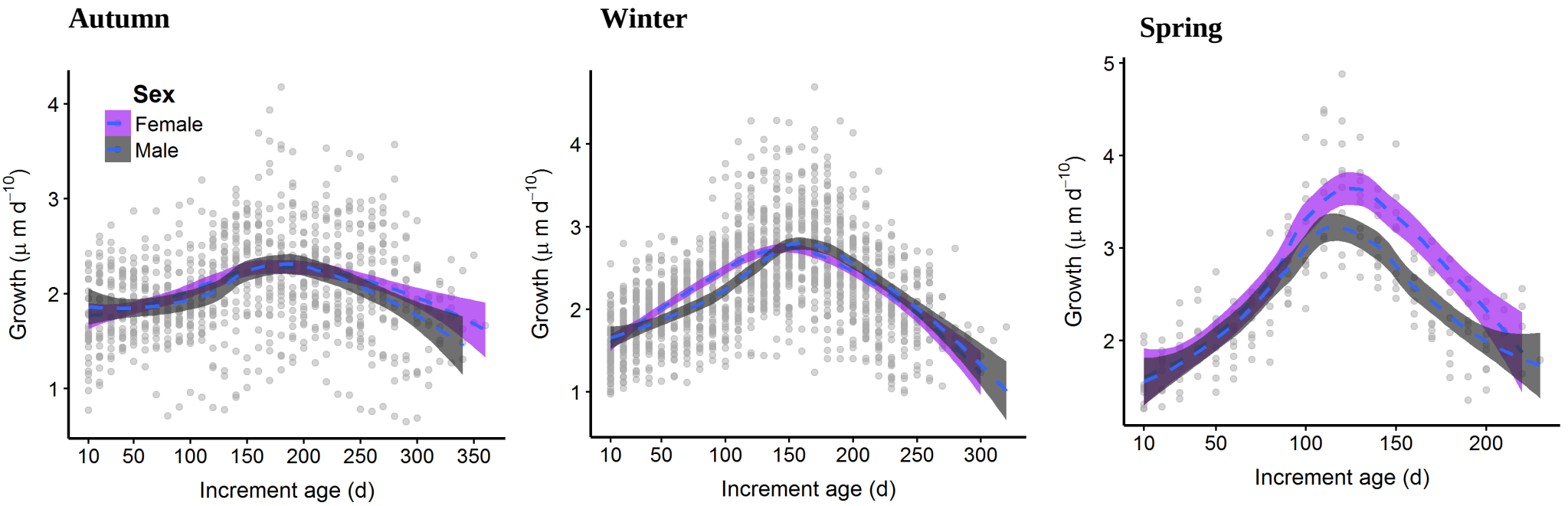


Figure 5.13. Age-dependent somatic growth trajectories of males and females within each hatch-season (autumn, winter and spring). The raw increment measurements are shown as points. A LOESS smother is fitted to visualise non-linear trend in somatic growth. The width of each LOWESS band is ± 1. S.E of the LOWESS smoother.

5.5 Discussion

In this study, the lifetime somatic growth trajectories of adult inanga were reconstructed from their otoliths, along with age and hatch date estimates. Considerable life history variation was found among adult inanga that was related to underlying differences in their somatic growth rates. Comparisons of age-dependent growth trajectories among hatch-seasons showed that larval-pelagic and adult-freshwater growth varied considerably among autumn, winter and spring-hatched fish. Significant differences in age at maturity were found among hatch seasons. However, no differences in size at maturity or reproductive investment were found. Relationships between variable growth trajectories coincident with hatch-season, size and age at maturity, along with reproductive output are complex, highlighting the dynamic nature of the life histories of inanga.

5.5.1 Demographic measurements

5.5.1.1 Age

Inanga were aged using daily ring counts in their otoliths. Aged fish spanned a wide range of sizes, but fish greater than one year old were not found. The oldest fish aged in this study was a 350 d summer-hatched male that was 76.3 mm L_T, whereas the largest fish aged was a summer-hatched female that was 95.1 mm L_T and 339 d. The age estimates in this study are largely consistent with previous assertions that inanga are an annual species (McDowall 1968, Laurenson et al. 2012). Chapman et al. (2006) found, however, that landlocked populations of inanga in Australia can live up to four years and are approximately 90 mm L_T at this age. The considerable disparity in size-at-age estimates between this study and Chapman et al. (2006) might be related to life-history differences between migratory and landlocked populations. Methodological differences between Chapman et al. (2006) and this study (growth zones versus daily ring counts) further mean that the results are not directly comparable. However, Chapman et al. (2006) also suggest that riverine populations in Australia are dominated by fish younger than one year old, based on their size-frequency distributions.

In my study, larger fish (>100 mm L_T) were present in samples, but their ages could not be derived because of difficulties during otolith preparation. These large fish made up < 4% of all fish sampled, and do not generally make up a large proportion of populations in other rivers and regions in New Zealand (McDowall 1968, Stevens et al. 2016). The age estimates in this

study are, therefore, representative of the demographics of populations in Golden Bay, at least in the studied rivers. Females > 100 mm L_T are considered to be two years old (Stevens et al. 2016), but size-age correlations for males and females indicate there is some decoupling between body-size and age. Some individuals are faster or slower growing for their age so that a large body-size does not necessarily imply an older fish.

5.5.1.2 Hatch dates

Back-calculated hatch dates provided further evidence (as in Chapter 2) that inanga spawning occurs during at least eight months of the year, from February through September. Summer and spring spawning events are not widely documented among populations in New Zealand (Taylor 2002). However, summer spawning is prevalent in New Zealand at higher latitudes (Hicks et al. 2013), whereas spring spawning is found at lower latitudes (Graham 1956). Summer- and spring-hatched fish were not identified in Golden Bay probably because they were not migrating inwards during September, October or November when sampling was done (see Chapter 2). Based on the strong association between migration timing and hatch dates, summer-hatched fish likely migrated inwards during the winter-months (July and August), while spring-hatched fish probably arrived in late-winter or early-spring (November and December). From the gonad development staging, recently spent fish were identified in February, confirming that spawning does occur in Golden Bay during the summer months. Spring spawning in Golden Bay is likely, but was not identified in this study.

5.5.2 Developmental trajectory

Among adults, distinct developmental transitions were evident from changes in age-dependent somatic growth rates. Pelagic and freshwater growth was separated by a period of peak increment widths that showed a marked plateau. This plateau represents metamorphosis from the post-larval to the juvenile form and additional juvenile growth. An extended period of slow growth is characteristic of metamorphosis in fish (Correia et al. 2003, Schismenou et al. 2016), and has been validated as the metamorphosis zone in other amphidromous species (Shen and Tzeng 2002). Hale and Swearer (2008) showed that growth increments prior to inward migration of inanga were narrower than those formed during ‘settlement’. Coupled with otolith microchemistry analysis, they showed that these changes in growth increments provide a relatively accurate measure of migration timing for inanga (Hale and Swearer 2008). Previously, I showed that somatic growth rates of post-larvae prior to inward migration were narrower (growth was slower) than those at the point of river entry (see Chapter 3).

Furthermore, I showed that the average age of post-larvae at inward migration aligned closely with the transition to the growth plateau in adults (see Fig. 5.7). These results further support that otolith microstructure patterns can be used to demarcate between pelagic and freshwater life phases for inanga.

Growth during the pelagic-larval phase showed similar age-dependent relationships to inward migrating post-larvae (see Chapter 3). Inflection points were found that likely represent the transition from the dispersive larvae to the more competent post-larval form. A steep increase in growth prior to inward migration was also obvious and is consistent with increasing growth prior to inward migration (see Chapter 3). A non-linear effect of growth with age following metamorphosis/juvenile-growth demarcates adult growth in freshwater. A second phase of declining growth was identified at an average age of 263 d. This decline likely coincides with the reallocation of energy from somatic-growth to maturation and reproduction (Wells et al. 2003).

5.5.3 Role of environmental variation on growth

The results of my study provide indirect evidence that temporal variation in environmental conditions coincident with hatching time affects the lifetime growth of inanga. Many studies show variable growth patterns among hatching times that are driven by temporal variation in temperature, food, weather conditions etc. (Alemany et al. 2006, Baumann et al. 2006, Weber et al. 2015, Schismenou et al. 2016). Winter-hatched and spring-hatched-fish were faster growing as pelagic larvae, younger at inward migration, and completed metamorphosis progressively quicker with lower age-dependent growth rates during their freshwater phase than autumn-hatched fish. By comparison, autumn-hatched fish were slower growing as pelagic-larvae, older at inward migration and took longer to complete metamorphosis but had higher age-dependent growth rates in freshwater.

Seasonal variability in sea surface temperature and primary productivity likely underpins the different pelagic growth rates found among hatching-times. Metamorphosis duration in inanga is likely affected by temperature and can have varying influences depending on the timing of habitat/life-stage transitions. For example, Fitzhugh et al. (1997) showed that Atlantic menhaden (*Brevoortia tyrannus*) recruiting to estuaries early in the season, during colder water temperatures, were slower growing during metamorphosis relative to those recruiting later during elevated temperatures. In New Zealand, river temperature is related to flow, solar radiation and air temperatures that vary seasonally (Jowett and Duncan 1990).

Temporal variation in river temperature is associated with the abundance of inward migrating glass eels (August and Hicks 2008) and also inanga (McDowall 1968), but less is generally known about the effects of temperature on metamorphosis. Because autumn-hatched fish mostly migrate during the early-spring, whereas winter-hatched and spring-hatched fish migrate in late-spring/early-summer, these patterns are consistent with temperature being the primary abiotic factor influencing metamorphosis in inanga.

During their freshwater phase, declining growth rates were found. This decline was steepest for later-hatched fish. Declines in growth occurred at an earlier age in spring-hatched fish (~140 d) compared to autumn-hatched fish (~ 200 d). For winter-hatched fish, declining growth rates were evident at 170 d. This pattern likely coincides with decreasing water temperatures from summer to autumn months. Similar patterns are seen in sprat (*Sprattus sprattus*), whereby later-hatched fish have sequentially steeper increases in larval growth and steeper declines in juvenile growth compared to earlier hatched fish, which is driven by seasonal changes in water temperature (Baumann et al. 2006). There was further evidence for the effects of abiotic conditions on growth because day influenced the growth trajectory of winter-hatched fish. This effect, however, was not found for autumn-hatched fish and the model for spring-hatched-fish failed to converge. The effect of day likely reflects the shorter growing season for winter-hatched fish; they must accumulate sufficient energy reserves prior to reproduction or else risk spawning at sub-optimal times, over-wintering in the river or not spawning at all. Photoperiod is one of the most important abiotic or "priming factors" that synchronises a fish's circadian rhythm, with seasonal changes in the abiotic environment (McCormick et al. 1998). This helps cue the physiological preparation for seaward migration, the onset of migration as well as sexual maturation in diadromous fishes (McCormick et al. 1998, Sykes et al. 2009). Seasonal differences in reproductive activity and latitudinal variation in spawning times show that photoperiod is important for regulating reproduction in inanga (Taylor 2002, Boy et al. 2009b, Stevens et al. 2016), and likely acts to synchronise the allocation of growth to reproduction in this species.

Adult inanga were sampled from rivers that varied widely in their characteristics such as catchment size, land use and flow (see Chapter 1). Yet river-specific differences were a lesser source of variation in growth than inter-and intra-individual differences among fish. Somatic growth was not explicitly related to biotic- and abiotic-conditions in the marine and freshwater environments. Such studies are challenging to undertake for migratory species because their specific environmental histories are difficult to define, especially in widely dispersing larvae. Often, large-scale environmental synchronisers acting on growth (e.g.,

climate or seasonality) of migratory fishes are more readily detected than more subtle fluctuations in abiotic conditions (Frederiksen et al. 2014) between individual rivers.

5.5.4 Relationships between pelagic and freshwater growth

Autocorrelation among growth increments demonstrates the influence that past conditions have on subsequent growth. This has obvious implications for understanding growth-environment relationships in fish (Pepin et al. 2001). For example, in larval Atlantic mackerel (*Scomber scombrus*), significant autocorrelation among daily increments indicates larval growth in the first two weeks of life affects later growth, which subsequently produced inter-annual recruitment variability (Robert et al. 2014). For inanga, growth in the first 50 d in the pelagic environment is auto-correlated. Processes occurring in the first two months of larval life might, therefore, be the critical link to understanding recruitment variations in this species. The lack of auto-correlation between growth during the first 50 d of pelagic life and increments at later stages suggests that inanga are growing in different environments from 50 d onwards. Friedland et al. (1996) showed that winter growth in Atlantic salmon (*S. salar*) was not correlated with summer or spring growth, which indicated that individuals were rearing in different environments or regions. In Chapter 3, I found a significant interaction between growth during larval life and age at inward migration. I hypothesized that once a growth rate/size threshold was attained, inanga move into alternative habitats such as the freshwater plume or coastal environments for a significant portion of their life prior to inward migration. The auto-correlation patterns among growth increments in adults provides a further line of evidence for a distinct switch in their growth coincidental with a life stage and habitat transition. Auto-correlation in somatic growth rates can override or mask the effects of extrinsic factors on growth (Hinrichsen et al. 2010) and is important to take into account for future studies.

Divergent growth trajectories were maintained among hatching times during freshwater growth but, interestingly, the magnitude and direction of growth was reversed to some extent. Autumn-hatched fish were slower growing during their pelagic phase, but they maintained higher growth rates for a longer period in freshwater compared to winter- and spring-hatched fish. Winter- and spring-hatched fish were much faster growing in the pelagic environment but slower growing in freshwater. This is one of the most novel findings of my study and provides the first critical insight into potential relationships between pelagic and freshwater growth histories in an amphidromous species. Nonetheless, the mechanisms underlying the divergent growth trajectories are unknown and there are likely many. One such mechanism for these

divergent trajectories is that autumn-hatched fish enter freshwater up to six weeks before winter- and spring-hatched fish. Consequently, they have greater access to the more productive resources and can take advantage of an extended period of growth in freshwater. This might be advantageous as autumn-hatched fish can compensate for their slower pelagic growth rates compared to winter- and spring-hatched post-larvae. However, temporal variation in abiotic conditions in rivers, particularly temperature and food availability can limit growth of autumn-hatched fish and so earlier arrival into rivers might not always confer a growth advantage.

Among anadromous fishes, the relationship between larval and juvenile growth determined in freshwater and adult growth in the marine environment are complex and highly variable. For example, freshwater growth and size at emigration has a limited influence on marine growth in Atlantic salmon (*S. salar*) populations, but growth during the first month at sea affects growth in the subsequent season (Hogan and Friedland 2010). Additionally, growth rates during the first and second sea winters were not related in Atlantic salmon indicating that compensatory growth occurs (Hogan and Friedland 2010), whereby fish that were initially slower growing can undergo a period of rapid growth so that their growth exceeds that of fish that were initially larger with higher growth rates (Ali et al. 2003). Marco-Rius et al. (2012) found a similar 'uncoupling' of freshwater and marine growth (as measured by body size) in populations of sea trout (*S. trutta*). However, other studies report significant relationships between freshwater and marine growth such as in sockeye salmon (*Oncorhynchus nerka*), whereby larger juveniles at outward migration grew more rapidly immediately on entry to the sea and this rapid growth carried through until their return to natal rivers (Freshwater et al. 2015). My results are discussed in more detail in the general discussion (see Chapter 6).

5.5.5 Reproduction and life histories

Relationships between variable growth trajectories associated with hatch-season, size and age at maturity along with reproductive investment are complex, highlighting the dynamic nature of the life histories of inanga. Significant differences in age at sexual maturity were found among hatch-seasons with autumn-hatched fish being older at sexual maturity than winter-hatched fish. Irrespective of the underlying differences in growth rates throughout development, autumn- and winter-hatched adults were similar sizes at sexual maturity. Size-age correlations indicate that larger mature autumn-hatched fish were older whereas mature winter-hatched fish had more variable ages for a given size. It appears then that size at sexual maturity for winter-hatched inanga is constrained and that adults must reach a size threshold

for maturity. This is not the case for autumn-hatched fish because smaller and younger adults were mature but larger and older adults were also mature. As discussed previously, the growing season of winter-hatched fish is more constrained compared with autumn because of the effects of day on growth. It is, therefore, likely that there are selective pressures acting on winter-hatched fish to be faster growing during the pelagic phase and to show rapid decreased growth during adult freshwater phase so that they can attain a certain size threshold for maturity.

The life history strategy of amphidromous fishes involves maximizing fecundity (Closs et al. 2013), and one way to do this is to maximize size at sexual maturity (Wootton 1998). Relationships between reproductive investment and size were significant and positive for winter-hatched fish but this was not seen for autumn-hatched fish. There are several potential reasons for this. One explanation is that autumn-hatched fish might delay the allocation of energy from somatic-growth to reproductive tissues for a longer period so they can maximise body size at spawning. This strategy is found among conspecific Chilean populations, whereby inanga spend longer allocating energy to somatic growth at the expense of reproductive investment (Boy et al. 2009a). Boy et al. (2009a) found that inanga spawn multiple times throughout the year, but ensure they have enough energy reserves to survive over winter. Recently, Stevens et al. (2016) found that some inanga survive spawning but it was uncertain if they spawned more than once. In this study, most mature autumn-hatched fish were sampled early in the season; but, some were immature and spent during this time. This shows that some autumn-hatched fish had spawned previously. Previous spawning may partially explain the non-significant relationships between gonad weight and size. It should be noted, however, that a limitation of this index is that macroscopic staging of gonads cannot fully ascertain an individual's previous reproductive history (Stevens et al. 2016). However, from the somatic-growth profiles of mature autumn-hatched fish, there was no indication of multiple growth stanzas that could be associated with multiple spawning events.

Closer inspection of previous work suggests complex relationships between growth/body size and reproduction are found among populations of inanga. For example, in Chilean populations, no relationship was found between fecundity and body size for females (Boy et al. 2009b), Australian populations show substantial variation in egg size within individual females (Semmens 2008), and McDowall (1968) further showed considerable variation in fecundity with increasing female body-size among New Zealand populations. Semmens and Swearer (2012) showed that inanga produce alternate larval phenotypes; larger larvae with smaller yolk sacs and smaller larvae with larger yolk sacs. They showed that the larval traits were not related to egg size, but are probably related to maternal investment

(Semmens and Swearer 2012). Although specific relationships for inanga were not derived, Closs et al. (2013) showed that among the amphidromous galaxiids, there is a trend of increasing egg size with larger body size. Collectively, this information indicates potential trade-offs between growth and various measures of reproduction in inanga. It is possible that the poor relationship between size and reproductive investment in autumn-hatched fish is related to underlying trades-offs between growth and reproduction. It is evident that life history pathways to maturity are widely different among hatch-seasons; thereby suggesting there are at least two different life history strategies pertaining to reproduction among inanga populations.

There was no evidence for differences between sexes in age-dependent somatic-growth rates among immature or mature fish within each hatch-season. A majority of studies have shown that males are often smaller at sexual maturity (Chapman et al. 2006, Barbee et al. 2011), mature earlier in the spawning season and have greater reproductive investment than females (Stevens et al. 2016). However, there is evidence to suggest that males and females have similar somatic growth rates (McDowall 1968). From the pool of total fish caught, mature males were significantly smaller than females (Fig. A.5.12). The average size of mature males that were aged was smaller than for mature females and so the growth reconstructions are representative of the size differences found between the sexes. The lack of differences between males and females may be due to differential energy allocation patterns between sexes that are not detected from otolith studies.

5.6 Conclusions

This is the first study to reconstruct the growth histories of adult inanga and to provide age estimates using otolith microstructure methods. A wealth of information about the lifetime growth of inanga and new insight into their ecology were gained. This study provides the first understanding of the relationships between pelagic and adult growth for an amphidromous species and suggests that the inanga can utilise pelagic and freshwater habitats to maximise growth and body size at maturity. Future studies that use otolith microchemistry methods to better define the timing of inward migration, coupled with back-calculated size estimates will lend a wealth of new insights into the migrations of inanga and other amphidromous species.

Chapter 6: General discussion

6.1 Overview

This thesis explored and integrated aspects of the early life history of inanga (*Galaxias maculatus)* with their inward migrations, population structure and adult life histories using otolith-based methods. I identified that processes occurring over various spatial and temporal scales drive differences in the life history of inanga. Asymmetries during early life indicate that regional variation in abiotic conditions were important factors shaping their pelagic life histories. Temporal variation in early life histories (ELH) showed that seasonality among other factors drive ELH as well as adult life history differences within and among regions. Regional patterns in ELH coupled with the physical and abiotic context of the larval-pelagic environment shape the dispersal trajectories and subsequent stock structure of this species, providing evidence that dispersal over ecologically relevant time scales is more constrained than previously known. This work provides several new insights into the biology and ecology of inanga with implications for fisheries management and conservation. The findings also contribute to the broader understanding of amphidromous life histories.

6.2 Divergent pressures drive variability in growth trajectories within and among populations

The life history strategies of species are driven by their environment. For species exposed to spatially and temporally heterogeneous environmental conditions, adaptation and phenotypic plasticity are central to understanding their life histories (Stearns 1992). Inanga experience heterogeneous conditions within and among populations throughout larval/post-larval, juvenile and adult development. Therefore, they are likely exposed to divergent selection pressures that can affect various stages of their life cycle. Among regions, significant differences in size and age at inward migration (that varied as a function of hatching time) (Chapter 2), pelagic larval stage durations and growth trajectories (Chapter 3) were found. Within regions, adult inanga showed significant differences in age-dependent growth rates and age at sexual maturity (Chapter 5). However, for the post-larval stages, and sexually mature adults, irrespective of underlying differences in age and growth rates, no differences in body size were found within regions. According to life history theory, the optimal life history strategy for a species is to

maximise lifetime reproduction, which can be achieved by maximising age-specific survival and fecundity (Stearns 1976). Growth rates are implicit to a species' life history strategy because growth determines the rate of increase in size for a given age (Arendt 1997). Therefore, variation in the trajectory of growth can produce body sizes at various developmental stages to maximise future fitness (Bertram et al. 1993) while increased growth rates can enlarge adult body size thereby increasing reproductive output (Wootton 1998). It is apparent that inanga are subjected to variable pressures during their lifetime because of the significant within and among region differences in their life histories. Consequently, substantial life-history plasticity can be found.

According to Cushing's match-mismatch hypothesis, the timing of spawning and development in fish are synchronised with conditions favourable for larval growth, survival and recruitment (Cushing 1990). However, spawning within and among inanga populations is not synchronised with favourable pelagic conditions (McDowall 1998). My work shows that the pressures acting on the growth rates of inanga to reach a certain body size for inward migration vary temporally, in line with hatching times. These temporal pressures vary in magnitude and direction and are likely related to the abiotic conditions experienced in each distinct habitat that inanga occupy during their life stages. During embryonic development, air temperatures decline over the course of the spawning season, and therefore early-spawned eggs experience different thermal conditions while developing in the riparian habitat compared to later-spawned eggs. As a result, embryonic development may be accelerated for early-spawned eggs relative to later-spawned eggs (Benzie 1968). In the pelagic environment, autumn-hatched larvae are likely to be released into conditions (temperatures, productivity) that are initially favourable for growth but which are gradually declining with declining day length. Conversely, winter-hatched larvae encounter conditions that are initially poor but which become increasingly favourable for growth with increasing day length (increasing productivity prior to spring blooms, increasing temperatures). Spring-hatched larvae are likely to encounter optimum conditions in the pelagic but face increasingly harsher conditions once they migrate to freshwater. In the freshwater environment, autumn-hatched post-larvae experience a longer period of increasingly more favourable conditions as productivity and temperature increase. Winter-hatched post-larvae that migrate inwards during November experience a shorter window of favourable growing conditions in rivers as the environmental conditions begin to decline with declining day length.

It is likely that faster pelagic growth, shorter stage durations and overall less time spent in the pelagic environment for winter-hatched larvae is adaptive because it ensures that larvae

reach a size refugia and minimise overwinter mortality (Arendt 1997). Likewise, slower growth, longer stage durations and older ages at inward migration of autumn-hatched fish ensures larvae do not grow too quickly in the pelagic environment thereby entering rivers during unfavourable winter conditions. Furthermore, steeper declines in adult freshwater growth for winter- and spring-hatched fish ensure that they can attain a size threshold for maturity and reproduction (Arendt 1997) while higher age-dependent growth rates for autumn-hatched fish means they can compensate for the slower growth in the pelagic environment and take advantage of the longer growing season in freshwater. Collectively these varying and opposing pressures partially drive the lifetime growth trajectories among hatching times for inanga.

Importantly, hatching time is a product of the integration of temporal variation in reproduction with variability in the biotic and abiotic habitats occupied during an individual's lifetime. It is therefore difficult to disentangle the specific mechanisms underlying growth trajectories. Temporal variation in growth trajectories during pelagic development of inanga may also be related to process occurring during reproduction and not solely driven by variation in the abiotic and biotic environment. Inanga may adopt strategies during reproduction to maximize larval survival given that the pelagic environment conditions are unpredictable and uncertain. For example, inanga may be able to hedge their bets via the production of variable larval phenotypes that differ from each other in their growth and dispersal related traits (Crean and Marshall 2009). Diversified bet-hedging, whereby females produce variable egg sizes (Semmens 2008) in increasingly unpredictable circumstances is found among populations of inanga in Australia (Morrongiello 2011).

In a study by Semmens and Swearer (2012), they found that inanga produce variable larval phenotypes: larger larvae with smaller yolk sacs and smaller larvae with larger yolk sacs. They showed that the larval traits were not related to egg size, but were probably related to maternal investment (Semmens and Swearer 2012). Whether egg size or larval traits vary in a directional manner throughout the spawning season is unknown. Furthermore, the demographic structure (Barbee et al. 2011), condition and/or reproductive history of spawning females (Stevens et al. 2016) might also affect egg size, offspring provisioning and thereby larval growth rates. Females could sacrifice fecundity and instead invest their energy in the production of larger offspring. Such size advantages at hatching might allow larvae to survive the period of most intense selective mortality in the plankton (Vigliola and Meekan 2002) which is likely greatest in winter with colder temperatures and lower food availability.

6.3 Dispersal is constrained but not uniform

Dispersal is defined as the movement of an individual away from its natal site and is regarded as one of the key life-history traits affecting the dynamics of populations (Clobert et al. 2009). For amphidromous species, pelagic larval dispersal has been the Achilles heel for understanding their life history. McDowall (2010) suggested that spending less time as a dispersive larva, and/or exploiting oceanographic features (e.g., eddies, gyres), were two potential mechanisms that could minimise larval dispersal but these mechanisms have not been tested. To further our understanding of the larval life histories of inanga, I inferred the extent of larval dispersal by integrating life history traits (larval growth rate, stage duration, size and age at inward migration) with known physical (speed and direction of the dominant ocean currents) and environmental features (temperature and productivity) of New Zealand's seascape. I further advanced McDowall's (2010) hypothesis by incorporating variation in hatching times, which enabled me to integrate adult reproductive dynamics with these seascape features. This 'bio-physical' approach (Treml et al. 2015) accounted for potential spatial differences in adult life histories because larvae enter the pelagic environment at different times of the year in each region which may influence and even restrict larval dispersal pathways (Fortier and Leggett 1983).

Inanga showed clear developmental stages during pelagic life: a long slow growth phase that represents the pelagic larval stage and a phase of rapid growth that represents the transition to a more competent post-larval stage. During the larval stage, growth rates were a significant driver of age at inward migration. Growth therefore determines the length of time inanga larvae spend in their larval duration stages whereby dispersal potential is greatest and determines the total amount of time spent in the pelagic habitat. Pelagic larval duration and post-larval competency are influenced by ambient conditions in the pelagic environment. Temperature is widely known to affect dispersal via its effects on growth and stage durations in fish (O'Connor et al. 2007) and is likely the primary abiotic factor constraining the larval duration stage and thereby length of time spent dispersing for inanga. Physical and environmental factors across New Zealand's seascape and among the studied regions underpins the spatial and temporal extent of dispersal for inanga which is mediated by larval growth rates. This result provides some of the first insights into mechanisms that constrain larval duration and thereby dispersive stages of inanga and aligns with an emerging theme of restricted dispersal among amphidromous species (Sorensen and Hobson 2005, Hogan et al. 2014, Warburton 2015).

Size/growth rate thresholds for inward migration identified in this study parallel studies on anadromous species which show seaward migration is driven by body size thresholds and associated growth rates during their early freshwater life (Acolas et al. 2012, Marco-Rius et al. 2013). One limitation of my study is that all post-larvae were assumed to have completed their pelagic phase in the marine environment and I discounted the possibility that non-migratory fish or those with alternative migratory histories (i.e., estuarine) were present in my samples. In other populations, freshwater larval development has been found but it was very rare (Hicks, 2012). Other studies have shown that larval development occurs exclusively in the marine environment; post-larvae migrate directly from the sea to freshwater with no evidence of an estuarine phase (Hicks et al. 2005). Nevertheless, understanding the factors that underlie these growth rate thresholds and the degree of genetic and phenotypic lability will lend important insights into the life histories of the migratory components of inanga and other amphidromous species. Furthermore, knowledge of size/growth thresholds will help with understanding drivers of partial migration and alternative migratory strategies that are widely found among amphidromous species (Augspurger et al. 2016).

6.4 Do the benefits of amphidromy vary among regions?

Latitudinal clines in life history traits of diadromous species can reflect the varying growth opportunities in freshwater and marine environments (Metcalfe and Thorpe 1990). For amphidromous species, these relationships are not widely understood. Most studies of amphidromous species are done in single rivers (Iida et al. 2008, Lejeune et al. 2016, Teichert et al. 2016) with few studies completed over wider spatial scales that incorporate spatial heterogeneity in the pelagic and freshwater environments. Inanga are an excellent model species to test the effects of abiotic gradients on their life histories because they are found from the Falkland Islands at 51 °S through to Victoria in Australia at 37 °S (McDowall 1990). Here, differences in size and age of inanga at inward migration, along with variable pelagic larval durations and age-related growth trajectories were found among regions that generally followed a latitudinal cline. Additional studies by Barbee et al. (2011) among Australian and New Zealand populations showed similar latitudinal differences in size and age at migration.

It is thought that the pelagic environment affords better growing opportunities for amphidromous species than freshwater (McDowall 2010). Therefore, it would be seemingly beneficial for inanga to extend their pelagic life to maximise growth and thereby body size before returning to freshwater. However, their migrations are driven by increased trophic

requirements and are thought to be synchronised with seasonal increases in productivity and growth opportunities in freshwater (McDowall 2010). Size and age at inward migration, and how this varies regionally, may therefore reflect different costs, benefits and trade-offs associated with growth opportunities in the pelagic and freshwater environments (Dingle and Drake 2007, Thorstad et al. 2016).

Regional variation in the abiotic marine environment in New Zealand is mostly driven by differences in depth, winter insolation, winter sea surface temperatures and tidal currents (Ministry for the Environment 2005) along with differences in primary productivity (Murphy et al. 2001). Furthermore, regional differences in river characteristics are found in New Zealand and distinct eco-regions can be defined (Ministry for the Environment 2010) based on significantly different climates, along with differences in topography, geology and land cover (Snelder and Biggs 2002, Snelder et al. 2005). Productivity in freshwater environments is generally greater in the North Island although there are many exceptions and land-use may override regional trends (Quinn and Hickey 1990). Nevertheless, regional comparisons of the life histories of inanga across abiotic gradients can potentially extend our understanding of amphidromy and the relative costs and benefits of migrating between marine and freshwater habitats. Recently, Górski et al. (2015) showed that the migratory behaviour of inanga in Chilean populations was influenced by riverine productivity. They found that in larger, warmer rivers with predictable flows and floodplain inundations, inanga do not undergo marine larval development but instead remain in the more stable, productive riverine habitat. Further south, freshwater habitats are subjected to greater hydrological variability due to higher precipitation, and are less productive. In these environments, Górski et al. (2015) found that most inanga developed in the marine environment. This is one of the first bodies of work suggesting that the migratory tendencies of an amphidromous species vary as a function of latitudinal differences in rainfall as a proxy for disturbance, temperature and productivity.

6.5 Whitebait fishery management and inanga conservation

Inanga are declining in New Zealand, mostly as a result of agricultural intensification and urbanisation that has compromised adult and spawning habitats (Goodman et al. 2014). The effects of fishing pressure on populations is especially contentious because of a lack of data combined with poor understanding of their life histories, demographics and population dynamics. These critical knowledge gaps have prevented a systematic and integrative approach to the management of the whitebait fishery in New Zealand which is currently managed as a

single biological unit (McDowall 1999). Implementing the most effective management strategy for the whitebait fishery is difficult because there are opposing drivers of management: maintaining abundance of the most dominant species in the catch versus diversity of all whitebait species.

Regional differences in the ELH and stock structure of inanga identified in this thesis suggest spatial management of the whitebait fishery is warranted, at the very least, at regional scales. Currently, two spatial management units are defined: the West Coast of the South Island and the rest of New Zealand (New Zealand Statutory Regulations 1994/65, 1994/66). Spatial management was designed to protect the 'rarer' whitebait species that are seemingly more prevalent on the West Coast of the South Island (McDowall 1991). However, this management framework makes no provisions for variation in the life histories and demographics of inanga, despite inanga comprising 88% of all fish caught in the whitebait fishery across the country (Yungnickel 2017). Recent studies suggest that the West Coast region does not have any greater proportions of the rarer galaxiid species than Waikato, Wellington and Tasman/Nelson (Yungnickel 2017). Given the findings of Yungnickel (2017) and the evidence of stock structure for inanga identified in this thesis, regional management could be improved on to achieve the dual goals of maintaining numbers of whitebait, life history diversity and species diversity. Sub-dividing the fishery into spatial management units would enable Department of Conservation (DOC) to impose region-specific restrictions on the fishery and also allow for an adaptive approach to fishery management where necessary.

Aside from spatial fishery management, consideration must be given to the scale of temporal management. Currently the fishing season is open from 15th September to 15th November on the West Coast of the South Island, while the rest of New Zealand has a fishing season from 1st September to 30th November (New Zealand Statutory Regulations 1994/65, 1994/66). From September to November, significant temporal differences in the pelagic life histories and migrations of inanga post-larvae were found. Slower growing autumn-hatched fish largely migrate in early spring (September) but faster growing winter-hatched fish migrate in late spring (November). These staggered migration times mean autumn- and winter-hatched fish may be exploited differentially because fishing pressure typically increases later in the season as the weather stabilises making fishing conditions more favourable. It is evident that the life history trajectories of inanga vary as a function of hatching time and protracted spawning likely serves to increase the "life history options" (Dingle and Drake 2007) available

to inanga. To maintain life history diversity, temporal management of the whitebait fishery must be considered.

Furthermore, adult growth trajectories in freshwater varied among hatch-seasons as did age at sexual maturity. Because winter-hatched fish spend less time growing in freshwater, they may be less susceptible to pressures in freshwater that can reduce survival relative to autumn-hatched fish that spend longer growing in rivers. Therefore, inanga with different hatch-dates, which imply different migration timings and life histories, may contribute to the dynamics and overall resilience of populations in different ways. Consequently, DOC must understand how the varying life histories of inanga are exploited and what the best options are to protect all components of populations are. Further evidence for a rethink on the temporal scale of management is shown by the significant numbers of juvenile inanga that have metamorphosed and are caught in the whitebait catch in November (Yungnickel 2017). These pigmented fish are summer- and autumn-hatched larvae that have arrived into rivers during July-September but are discarded and are considered as 'by-catch'. To protect these juveniles that have already survived the critical post-larval juvenile bottleneck, the fishing season should be curtailed to October for most parts of the country.

One critical knowledge gap is that relationships between reproductive output over the spawning season and contributions to the fishery are unknown. As such, there is little information about the contribution in terms of absolute numbers of autumn-and winter-hatched fish to the whitebait fishery. To help maintain the integrity of the whitebait fishery, the numbers of fish caught in any given river must not have significant negative effects on the numbers that survive to reproduce. The temporal extent of reproduction, along with reproductive output, are key drivers of fish demographics and population dynamics (Secor 2007). Ensuring egg and larval production occurs throughout the year is therefore critical to maintaining life history diversity and buffering inanga against stochastic processes that can vary spatially and temporally. Protracted spawning can lessen the effects of environmental variation on early life stages, dampening fluctuations in mortality and survival and thereby buffering population fluctuations (Secor 2007).

6.6 Conclusions

This is one of the first bodies of work to systematically reconstruct the life histories of an amphidromous species from their pelagic-larval life to their adult life in freshwaters. This thesis sheds new light on the life history of inanga highlighting the dynamic workings of this iconic

species. A better understanding of the ‘black box’ of pelagic development was gained and it is evident that this phase in the life cycle of inanga sets the scene for their life histories.

Appendix 1

A.1.1 Supplementary data

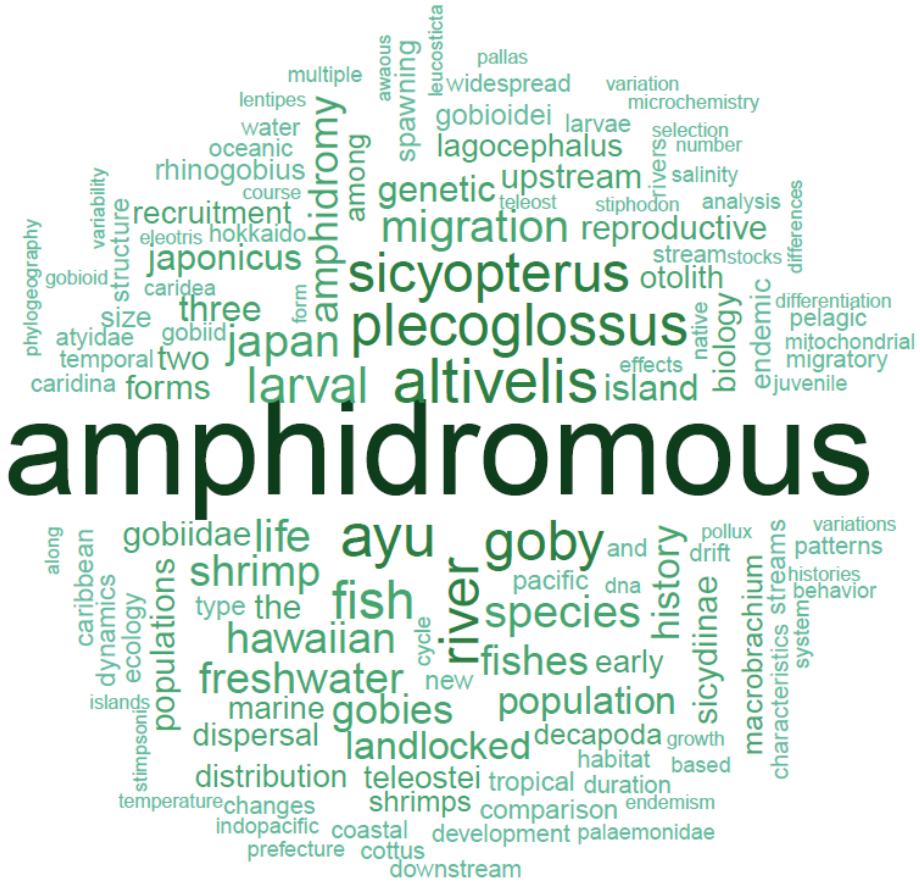


Figure A.1.1. Google scholar word cloud generated from search terms for amphidromous in the title of published works from the web of science database. Data accessed 7th January 2017.

A.2.1 Supplementary data

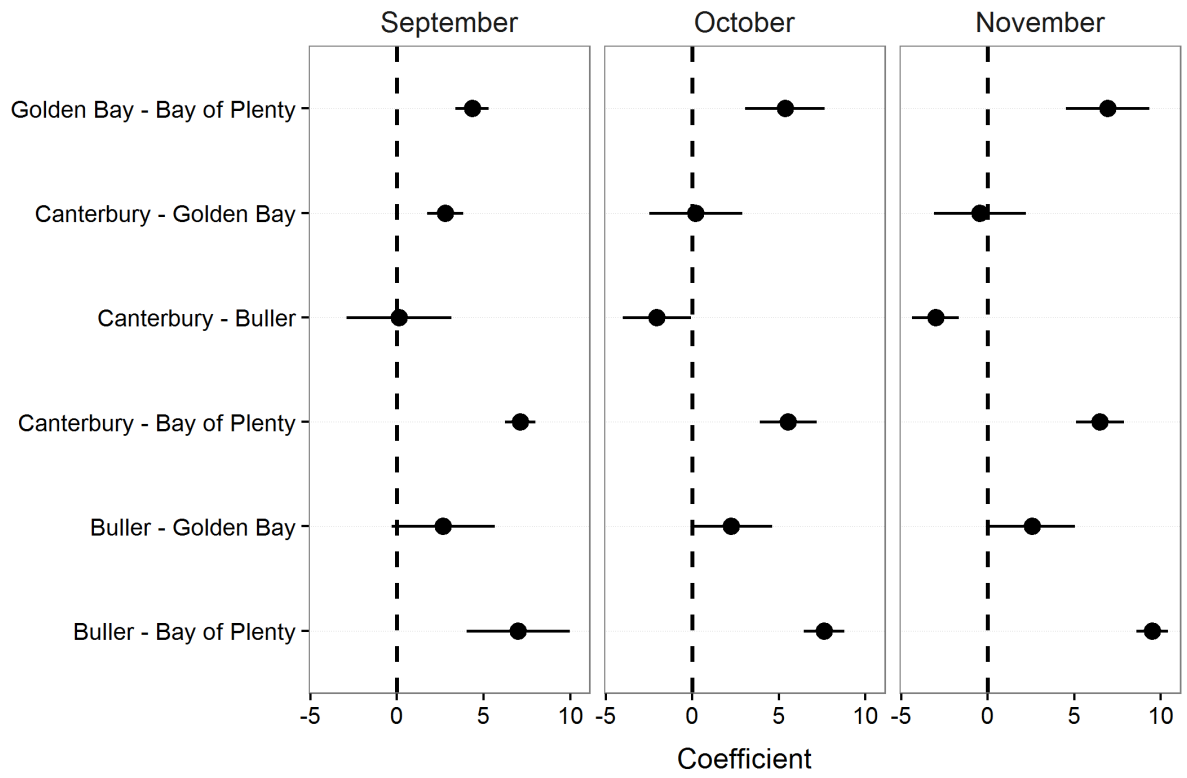


Figure A.2.1. Confidence intervals (95%) of Bonferroni corrected p-values for multiple comparisons of L_t among regions and $month_{migration.}$ Intervals that overlap zero (dashed line) indicate comparisons that are not significant.

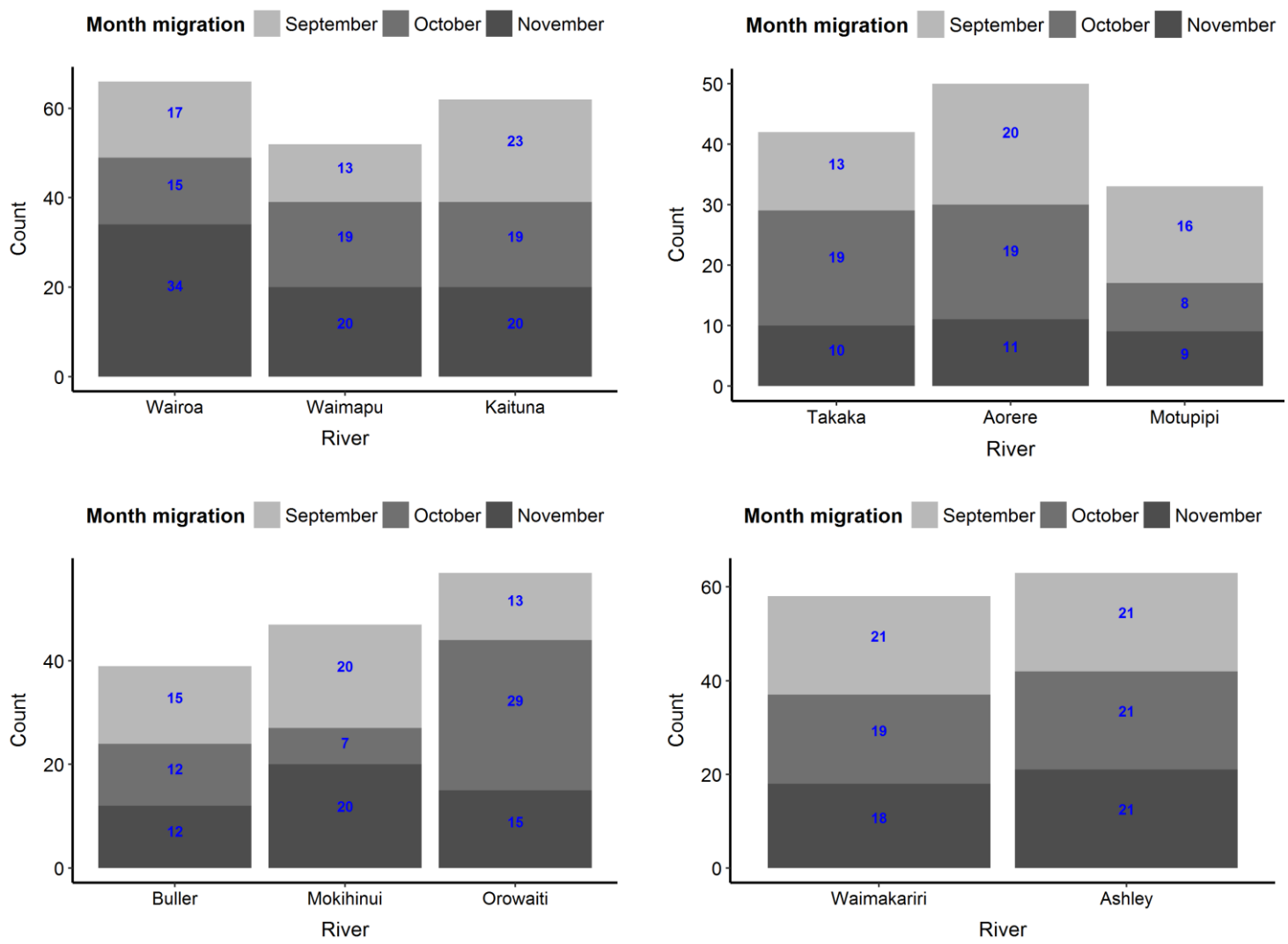


Figure A.2.2. Numbers of post-larvae that were aged with hatch dates obtained in each river among $month_{migration}$ within **a)** Bay of Plenty, **b)** Golden Bay, **c)** Buller and **d)** Canterbury regions.

Table A.2.1. Kolmogorov-Smirnov test results for differences in hatch-date distributions among regions. Significant differences are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS = not significant. D denotes the shape of the distribution.

Region		**Golden Bay**	**Buller**	**Canterbury**
Bay of Plenty		D = 0.6 ***	D = 0.7 ***	D = 0.6 ***
Golden Bay		-	D = 0.2 **	D = 0.2 **
Buller		-	-	D =0 .2 NS

Table A.2.2. Kolmogorov-Smirnov test results for differences in hatch-date distribution within regions among $month_{migration}$. Significant differences are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS = not significant.

Region	**$Month_{migration}$**		
		October	November
Bay of Plenty	September	D= 0.68 ***	D= 0.78 ***
	October	-	D = 0.43 ***
Golden Bay	September	D = 0.50 ***	D = 1 ***
	October	-	D = 0.91 ***
Buller	September	D = 0.57 ***	D = 0.8 ***
	October	-	D = 0.26 NS
Canterbury	September	D = 0.69 ***	D = 0.89 ***
	October	-	D = 0.59 ***

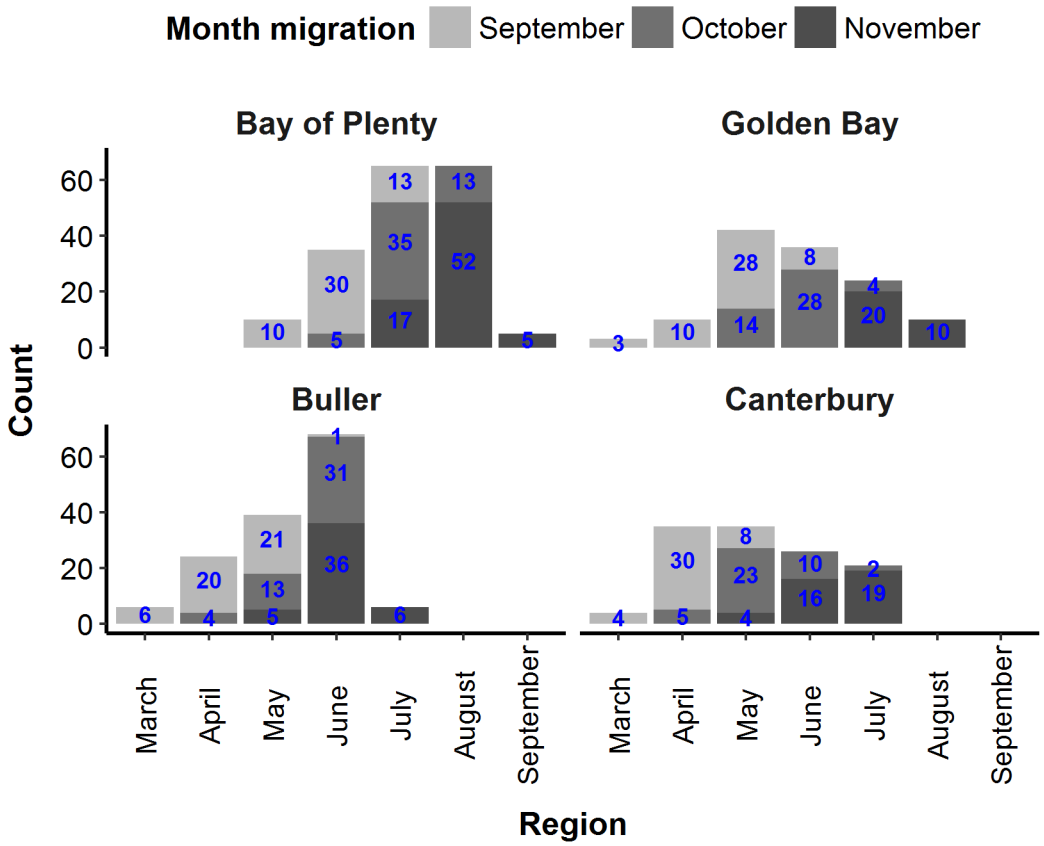


Figure A.2.3. Numbers of post-larvae in each hatch-month category among regions and $month_{migration}$.

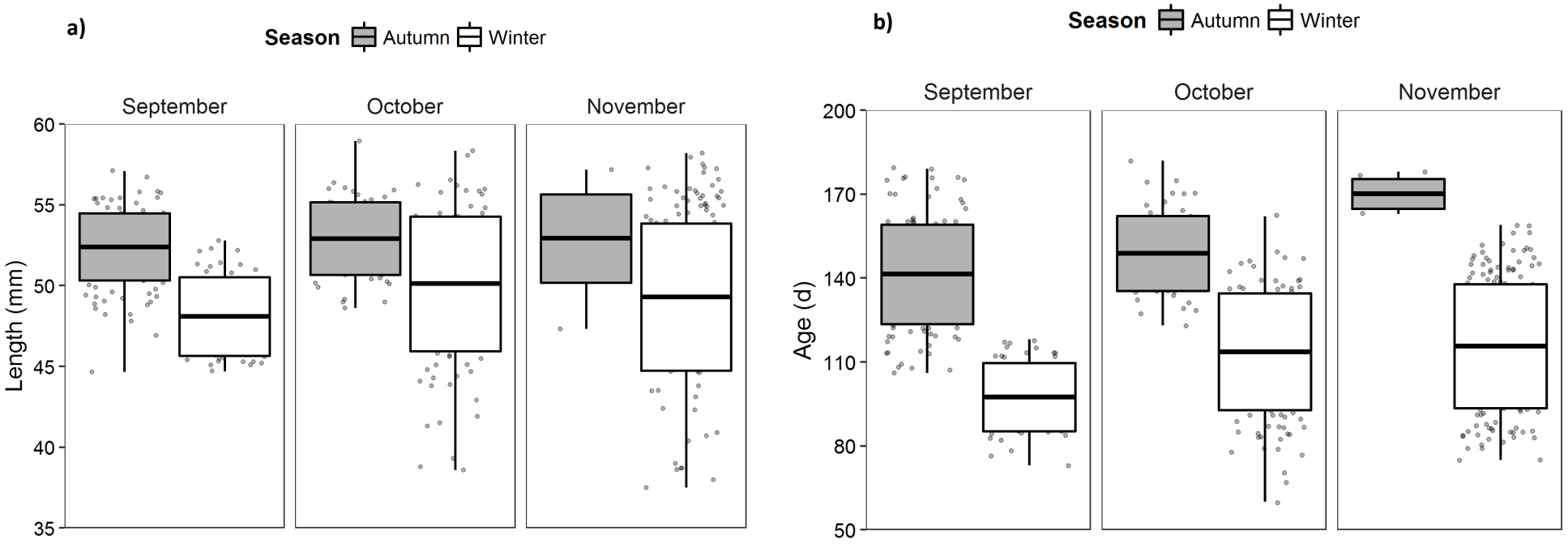


Figure A.2.4. Boxplots showing **a)** mean L_t and b) mean age (d) at inward migration of post-larvae within and between hatch-seasons and $month_{migration}$. Lines are the maximum and minimum values observed within each sample. The height of the boxes is the within-sample standard deviation.

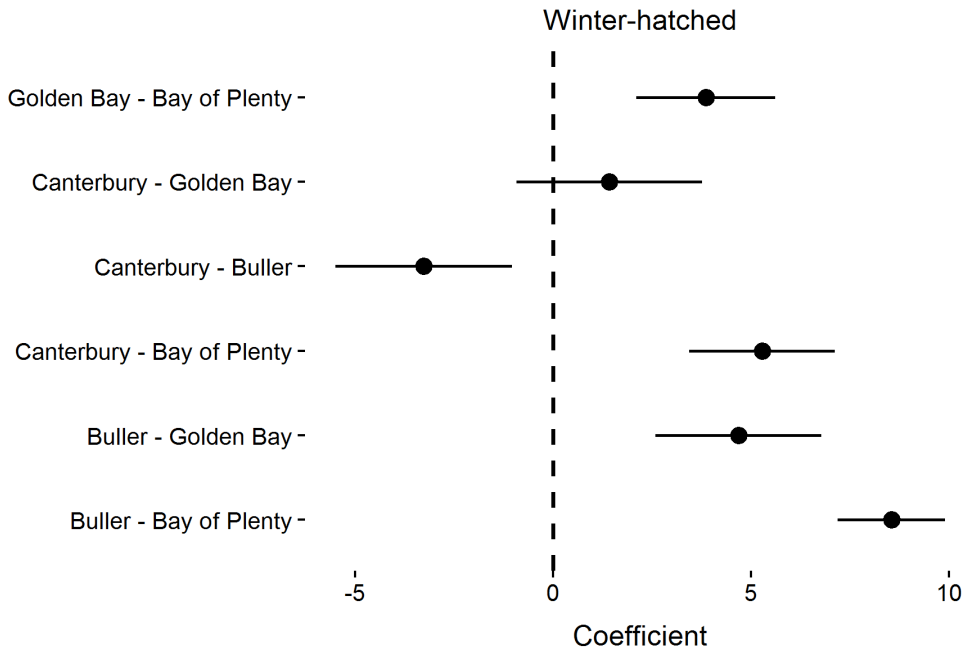


Figure A.2.5. Confidence intervals (95%) of Bonferroni corrected p-values for multiple comparisons of size of winter-hatched post-larvae between regions. Intervals that overlap zero (dashed line) indicate comparisons that are not significant.

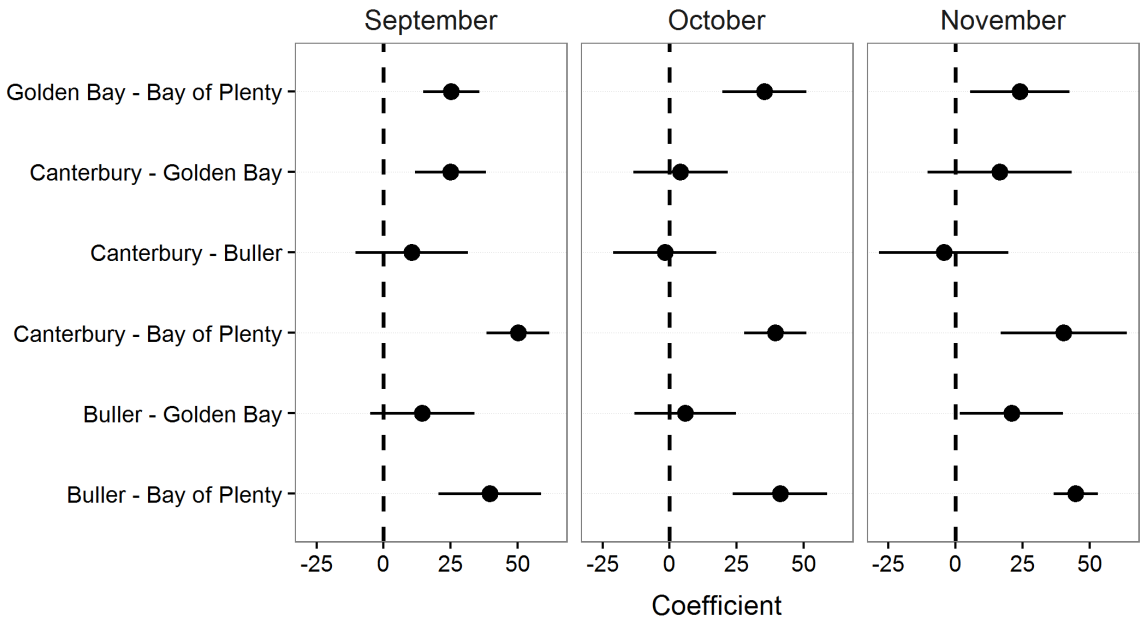


Figure A.2.6. Confidence intervals (95%) of Bonferroni corrected p-values for multiple comparisons of age (d) within and among regions and $month_{migration}$. Intervals that overlap zero (dashed line) indicate comparisons that are not significant.

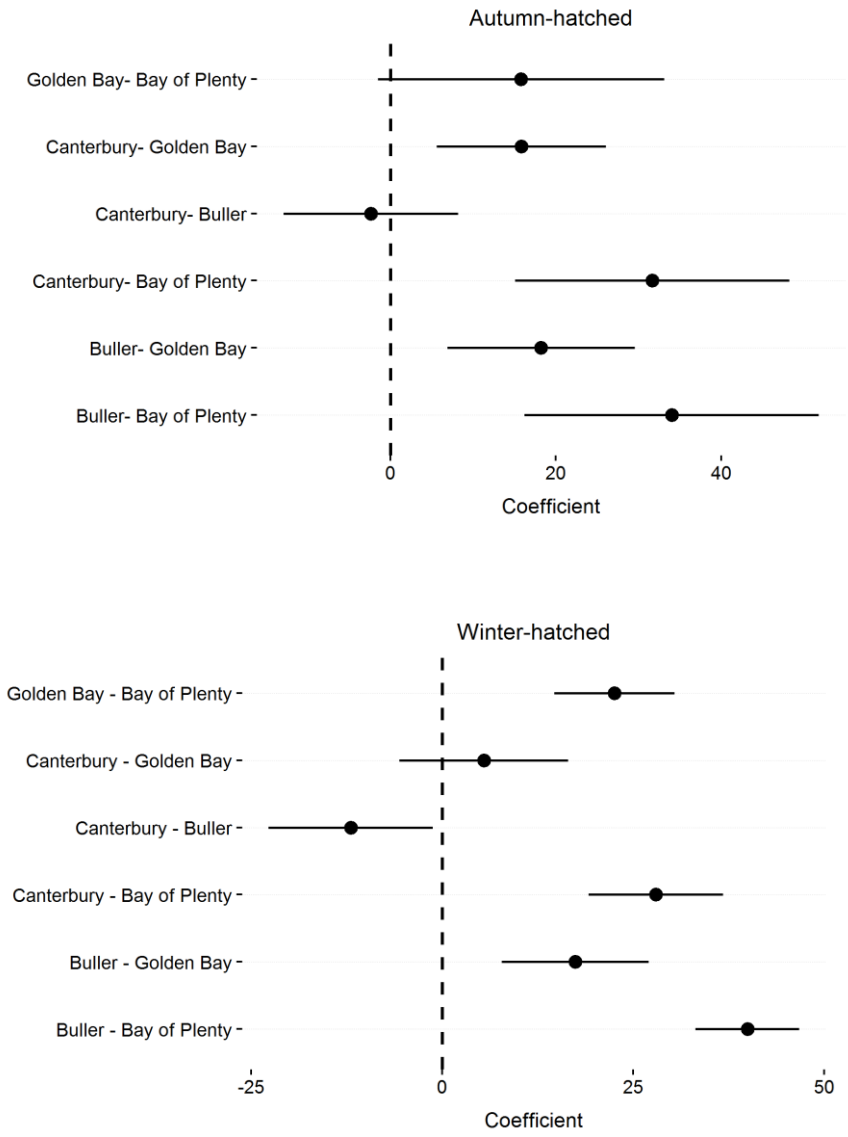


Figure A.2.7. Bonferroni corrected 95% confidence intervals for multiple comparisons among regions for differences in age at inward migration (d) among regions. Intervals that overlap zero (dashed line) indicate comparisons that are not significant.

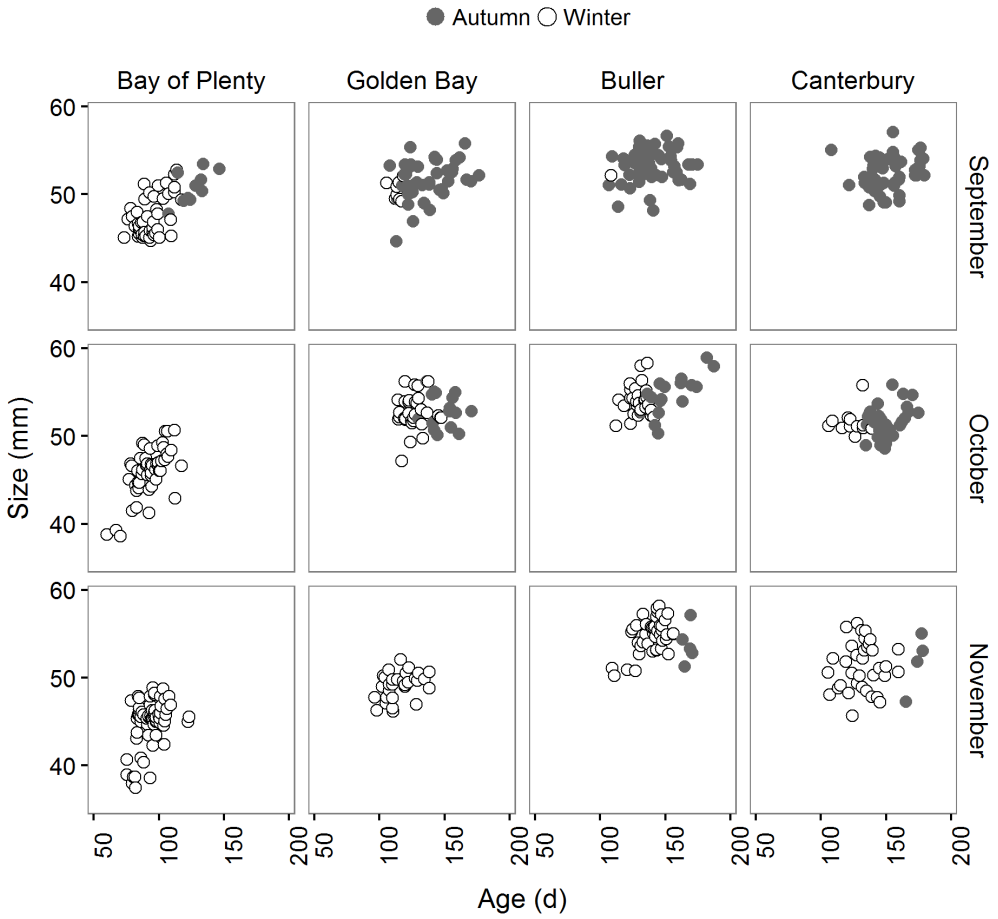


Figure A.2.8. Association between size and age at inward migration within regions and months according to hatch-season. Autumn-hatched post-larvae are dark grey circles and winter-hatched post-larvae white circles.

Appendix 3

A.3.1 Supplementary data

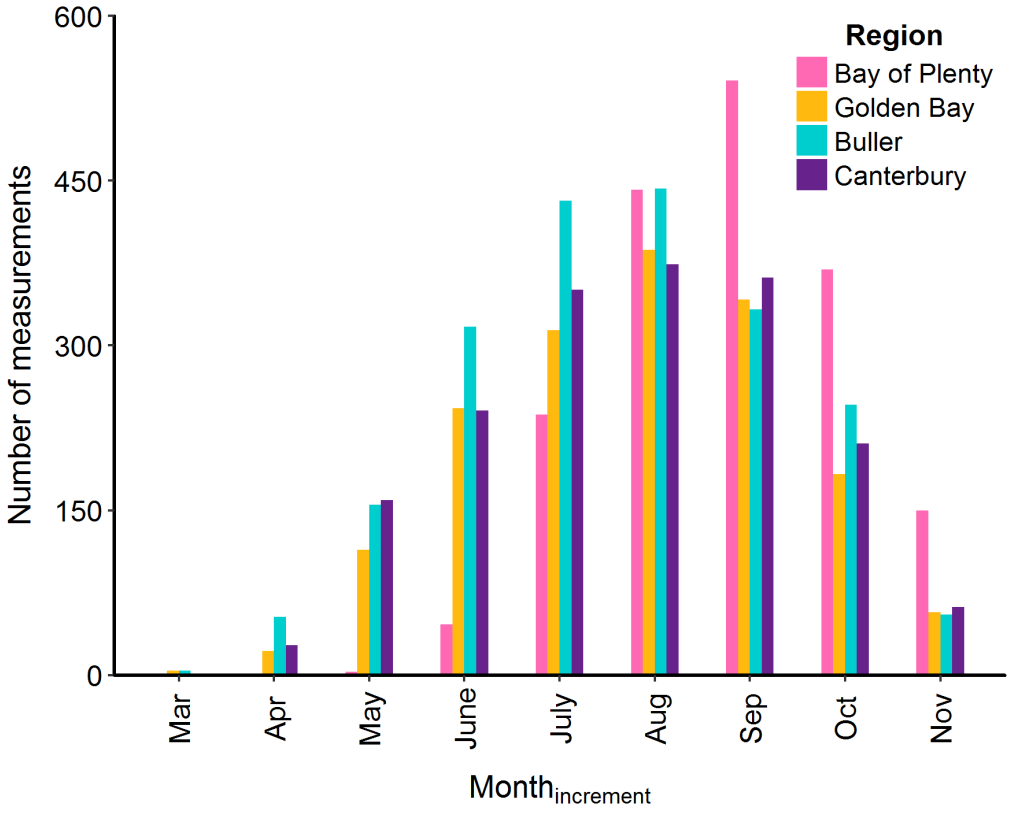


Figure A.3.1. Number of otolith increment measurements representing each month ($Month_{increment}$) for the temporally resolved "environmental proxy" for post-larvae in each region.

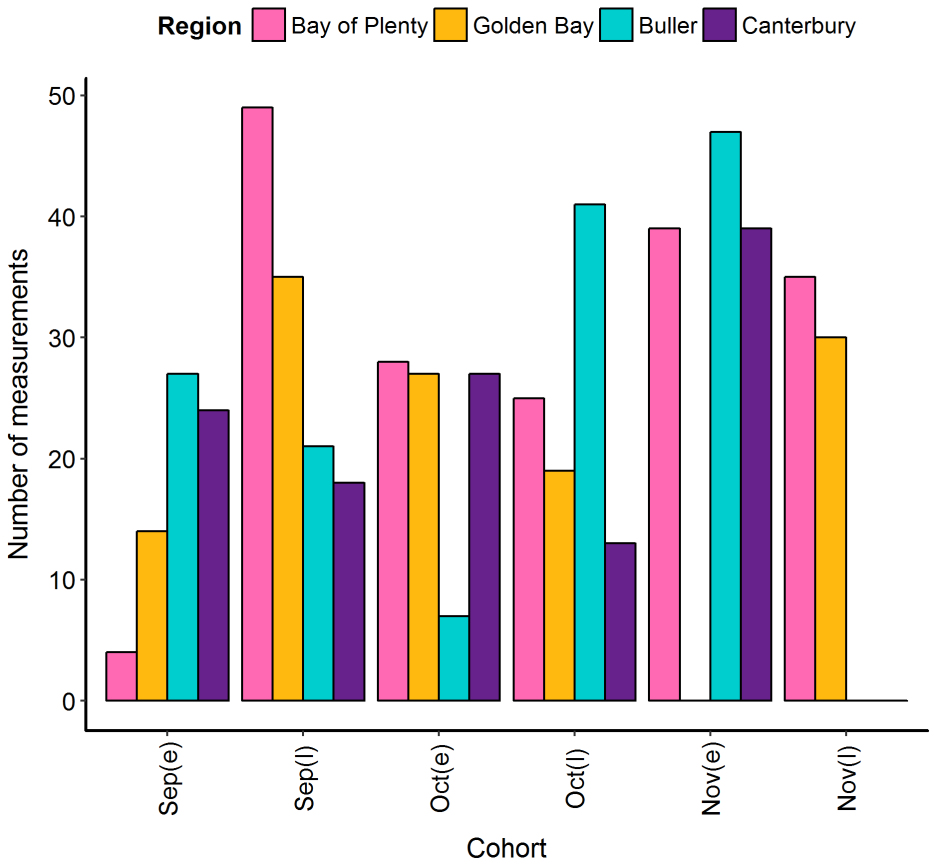


Figure A.3.2. Number of post-larvae from each respective cohort; e (early) and l (late) across $month_{migration}$

Table A.3.1. Numbers of inanga post-larvae used in reconstructing growth trajectories within and among regions.

Region	River	Month	n	Hatch-month	n	Mean age (± SD)	Cohort	n	Mean age (± SD)
Bay of Plenty	Wairoa	Sep	17	May	10	125.9 (11.1)	Sep (e)	4	102.75 (9.53)
	Waimapu		13	June	35	100.29 (8.7)	Sep (l)	49	99.96 (16.99)
	Kaituna		23	July	65	95.0 (9.9)	Oct (e)	28	94.18 (6.37)
	Wairoa	Oct	15	Aug	65	88.1 (9.4)	Oct (l)	25	90.64 (15.37)
	Waimapu		19	Sep	5	72.6 (9.4)	Nov (e)	39	92.64 (8.53)
	Kaituna		19				Nov (l)	35	91.82 (13.26)
	Wairoa	Nov	34						
	Waimapu		20						
	Kaituna		20						
Golden Bay	Aorere	Sep	20	Mar	3	169.0 (7.5)	Sep (e)	14	132.21 (17.55)
	Takaka		13	Apr	10	154.0 (8.2)	Sep (l)	35	133.74 (18.73)
	Motupipi		16	May	42	135.5 (15.0)	Oct (e)	27	132.74 (13.88)
	Aorere	Oct	19	June	36	124.6 (9.8)	Oct (l)	19	135.42 (15.92)
	Takaka		19	July	24	120.2 (8.8)	Nov (e)	-	-
	Motupipi		8	Aug	10	103.4 (3.8)	Nov (l)	30	115.23 (11.63)
	Aorere	Nov	11						
	Takaka		10						
	Motupipi		9						
Buller	Mokihinui	Sep	20	Mar	6	164.8 (7.5)	Sep (e)	27	139.96 (19.38)
	Orowaiti		13	Apr	24	148.9 (13.9)	Sep (l)	21	137.23 (14.42)
	Buller		15	May	39	137.4 (17.5)	Oct (e)	7	144.41 (17.10)
	Mokihinui	Oct	7	June	68	136.2 (10.2)	Oct (l)	41	135.32 (13.54)
	Orowaiti		29	July	6	119.5 (7.6)	Nov (e)	47	142.06 (13.64)
	Buller		12				Nov (l)	-	-
	Mokihinui	Nov	20						
	Orowaiti		15						
	Buller		12						
Canterbury	Ashley	Sep	21	Mar	4	177.5 (1.7)	Sep (e)	24	149.08 (15.42)
	Waimakariri		21	Apr	35	155.0 (11.2)	Sep (l)	18	154.39 (15.76)
	Ashley	Oct	21	May	35	146.9 (14.7)	Oct (e)	27	147.33 (14.26)
	Waimakariri		19	June	26	136.6 (10.8)	Oct (l)	13	133.15 (17.47)
	Ashley	Nov	21	July	21	120.8 (9.4)	Nov (e)	39	135.82 (18.09)
	Waimakariri		18				Nov (l)	-	-

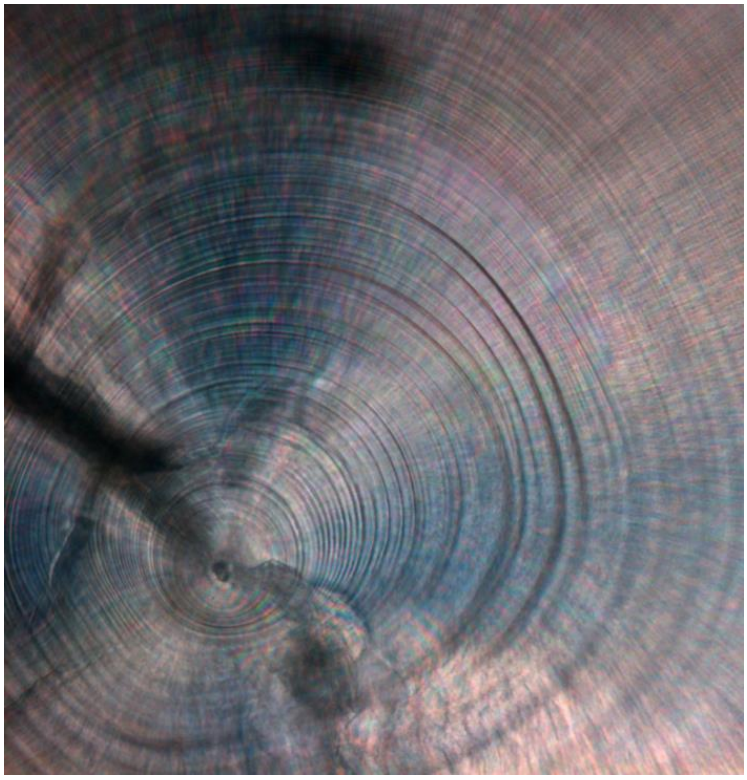


Figure A.3.3. Photo-micrograph at 40x magnification illustrating the complex microstructure observed in Canterbury fish. Multiple check marks can also be identified.

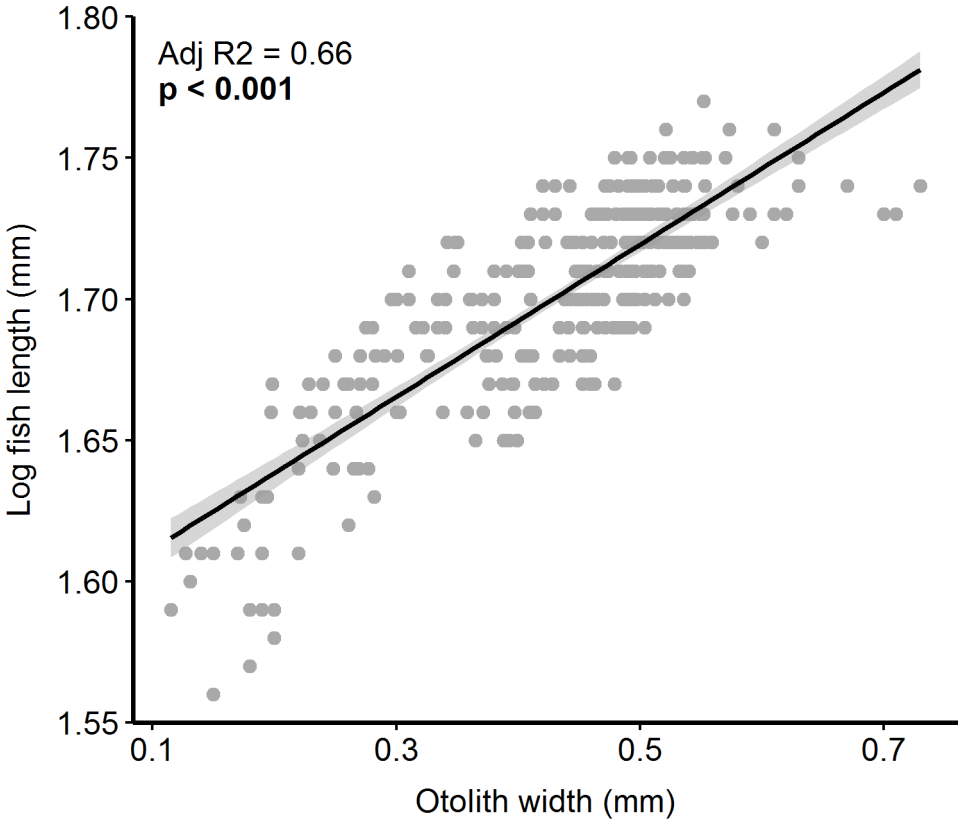


Figure A.3.4. Linear regression showing the relationship between fish-length and otolith-width for the post-larval stages of inanga.

Table A.3.2. Random intercepts (1|y) and growth slopes (x|y) for individuals (Fish_ID) within each region. Models are fitted with the intrinsic fixed effects $Age_{increment}$ + $Age_{migration}$. LL = log likelihood. K = model complexity. The optimal random effect models are highlighted in bold.

	Bay of Plenty				**Golden Bay**				**Buller**				**Canterbury**			
Random effects	**K**	**ΔAIC$_c$**	**AIC$_c$**	**LL**	**K**	**ΔAIC$_c$**	**AIC$_c$**	**LL**	**K**	**ΔAIC$_c$**	**AIC$_c$**	**LL**	**K**	**ΔAIC$_c$**	**AIC$_c$**	**LL**
$Age_{increment}$|Fish_ID	**7**	**0**	**-978.387**	**494.72**	**7**	**0**	**-910.15**	**462.11**	**7**	**0**	**-616.40**	**315.23**	**7**	**0**	**-866.35**	**501.35**
1|Fish_ID	5	189.43	-785.93	397.98	5	172.25	-737.90	373.97	5	64.89	-551.52	280.77	5	122.29	-988.64	438.19

Table A.3.3. Model selection results for optimal random slopes models for each region (where applicable). LL = log likelihood. K = model complexity. The optimal random effect models are highlighted in **bold**. M_i = $Month_{increment}$, H_m = $Hatch_{month}$, C = Cohort, A_i = $Age_{increment.}$ Each model is fitted with the optimal random intercept effects structure identified in Table 8 where relevant.

Region	**Random effects**	**K**	**ΔAIC$_c$**	**LL**	**AICc**
Bay of Plenty	**$1M_i + A_i\|C$**	**11**	**0**	**547.87**	**-1073.59**
	$1M_i + 1\|C$	9	31.07	530.31	-1042.52
Golden Bay	**$A_i\|M_i + 1\|H_m$**	**11**	**0**	**532.35**	**-1042.54**
	$1\|M_i + 1\|H_m$	9	22.01	519.32	-1020.53
	$1\|M_i + A_i\|H_m$	11	23.40	520.65	-1019.14

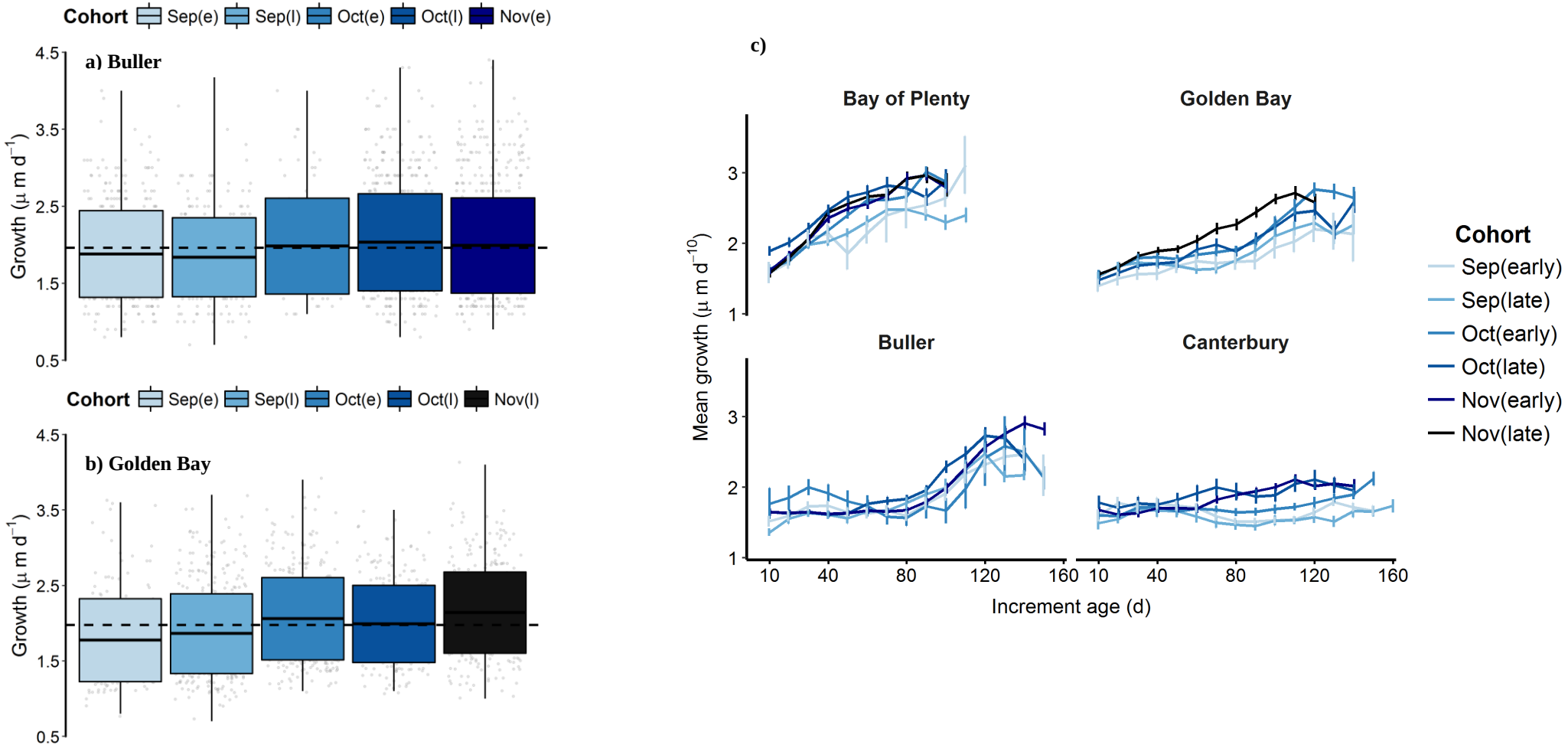


Figure A.3.5. Boxplots showing mean daily growth (μmd^{-1}) among early (e) and late (l) cohorts within **a)** Buller and **b)** Golden Bay. Lines are maximum and minimum daily increment widths within each region. Dashed line represents mean daily growth (μmd^{-1}) of all post-larvae in each region. Panel **c)** shows the age-dependent growth trajectories (± SE) among cohorts within each region.

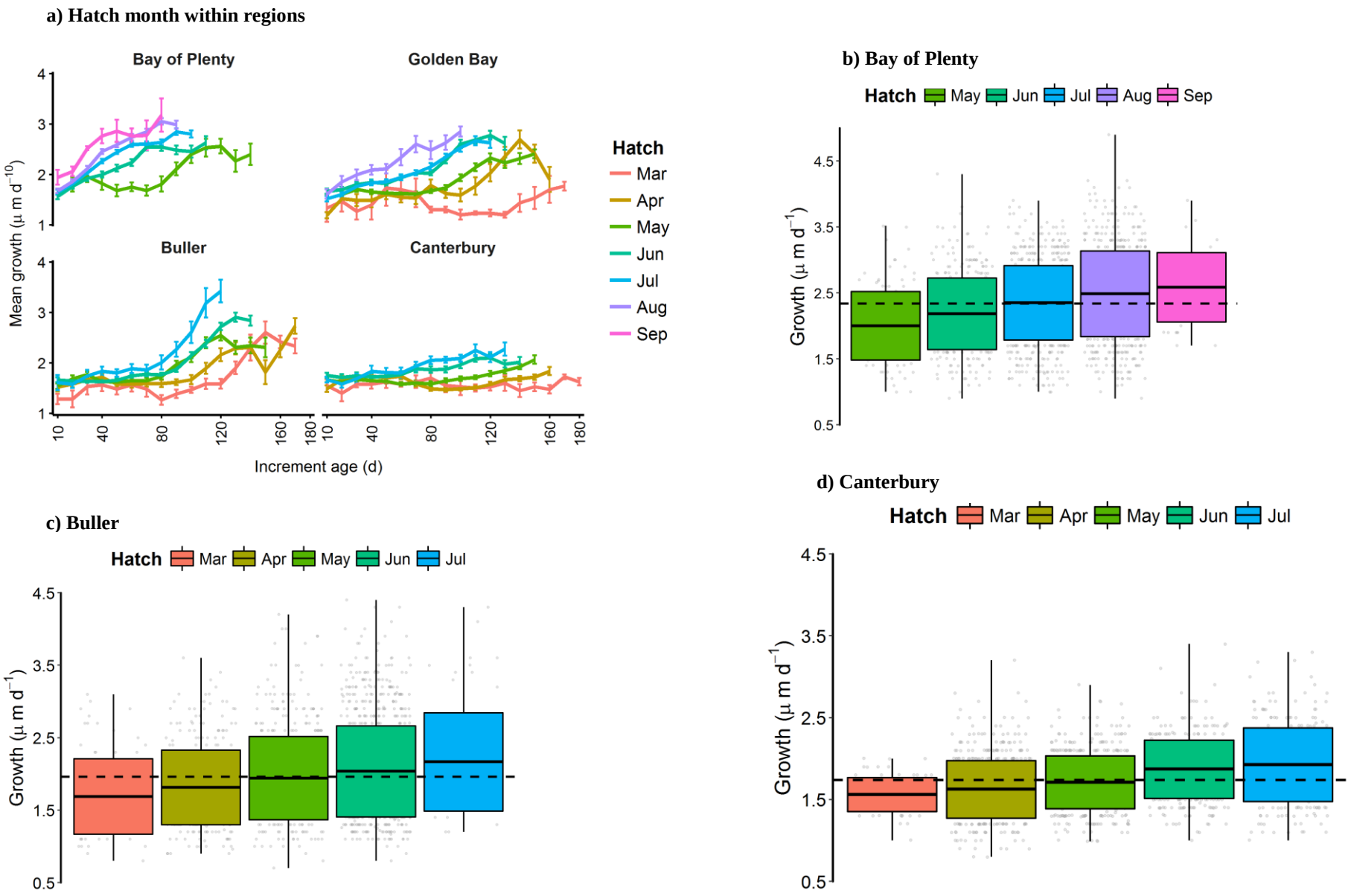


Figure A.3.6. Panel **a)** shows the age-dependent growth trajectories (± SE) among hatch months within each region. The boxplots show mean daily growth (µmd^{-1}) of post-larvae in each Hatch$_{months}$ within **a)** Bay of Plenty **b)** Buller and **c)** Canterbury regions. Lines are maximum and minimum daily increment widths within each region. The width of the boxes is ±1SD. Dashed line represents mean daily growth (µmd^{-1}) of all post-larvae in each region.

Table A.3.4. Intrinsic fixed effects model selection results for each region. * denotes interaction term. LL = log likelihood. K = model complexity. Optimal models are shown in bold.

Region	Intrinsic fixed effects	Model #	K	ΔAIC_c	AIC_c	LL
Bay of Plenty	**$Age_{increment}$*$Age_{migration}$**	**I1**	**14**	**0**	-1206.17	617.20
	$Age_{increment}$ + $Age_{migration}$	I2	13	4.06	-1202.11	614.16
	$Age_{increment}$	I3	12	84.58	-1121.59	572.88
Golden Bay	**$Age_{increment}$*$Age_{migration}$**	**I4**	**12**	**0**	**-1063.85**	**544.02**
	$Age_{increment}$ + $Age_{migration}$	I5	11	4.43	-1059.42	540.79
	$Age_{increment}$	I6	10	33.73	-1030.12	525.13
Buller	**$Age_{increment}$*$Age_{migration}$**	**I7**	**9**	**0**	**-931.81**	**474.95**
	$Age_{increment}$ + $Age_{migration}$	I8	8	8.8	-923.01	469.54
	$Age_{increment}$	I9	7	102.6	-829.21	421.63
Canterbury	**$Age_{increment}$ + $Age_{migration}$**	**I10**	**9**	**0**	**-1124.52**	**571.31**
	$Age_{increment}$*$Age_{migration}$	I11	10	0.32	-1124.20	572.16
	$Age_{increment}$	I12	8	34.81	-1089.71	552.89

Table A.3.5. Extrinsic fixed effect model selection results describing growth variation within each region. * denotes interaction term. LL = log likelihood. K = model complexity. The optimal models are highlighted in bold.

Region	Extrinsic effects	Model #	K	ΔAIC_c	AIC_c	LL
Bay of Plenty	$Age_{increment}$*$Age_{migration}$*River	E2	20	0	-1111.45	575.96
	$Age_{increment}$*$Age_{migration}$ + River	**E3**	**14**	**1.42**	**-1110.04**	**569.14**
	$Age_{increment}$*$Age_{migration}$	E4	12	4.87	-1106.58	565.38
Golden Bay	**$Age_{increment}$*$Age_{migration}$**	**E5**	**12**	**0**	**-1063.85**	**544.02**
	$Age_{increment}$*$Age_{migration}$*River	E6	14	0.8	-1063.05	545.65
	$Age_{increment}$*$Age_{migration}$ + River	E7	20	9.7	-1054.15	547.33
Buller	**$Age_{increment}$*$Age_{migration}$**	**E8**	**9**	**0**	**-931.81**	**474.95**
	$Age_{increment}$*$Age_{migration}$ + River	E9	11	3.33	-928.48	475.31
	$Age_{increment}$*$Age_{migration}$*River	E10	17	11.27	-920.54	477.42
Canterbury	**$Age_{increment}$ + River *$Age_{migration}$**	**E11**	**11**	**0**	**-1133.91**	**578.03**
	$Age_{increment}$ + $Age_{migration}$ + River	E12	10	4.16	-1129.74	574.93
	$Age_{increment}$*River + $Age_{migration}$	E13	11	5.26	-1128.65	575.40
	$Age_{increment}$ + $Age_{migration}$	E14	9	9.39	-1124.52	571.31

Table A.3.6. Model selection results for **a)** random intercept only models and **b)** nested random intercept models. Nested terms are denoted by colon (:). LL = log likelihood. K = model complexity. The optimal random effect models are highlighted in **bold**. A_i = $Age_{increment}$, C = Cohort, H_m = $Hatch_{month}$, M_i = $Month_{increment}$, R_g = Region.

a) Random intercepts						b) Nested random intercepts					
Model #	**Random effects**	**K**	**ΔAIC_c**	**LL**	**AIC_c**	**Model #**	**Random effects**	**K**	**ΔAIC_c**	**LL**	**AIC_c**
R1	**$1\|R_g + 1\|H_m + 1\|C + 1\|M_i$**	**11**	**0**	**1833.80**	**-3645.55**	**R1a**	**$1\|R_g + 1\|R_g{:}H_m + 1\|R_g{:}M_i + 1\|C$**	**11**	**0**	**1984.74**	**-3947.45**
R2	$1\|H_m + 1\|C + 1\|M_i$	10	17.92	1823.83	-3627.64	R1b	$1\|R_g + 1\|R_g{:}H_m + 1\|R_g{:}M_i + 1\|R_g{:}C$	11	5.57	1981.96	-3941.88
R3	$1\|C + 1\|M_i$	9	19.54	1822.02	-3626.01	R1c	$1\|R_g + 1\|H_m + 1\|R_g{:}M_i + 1\|R_g{:}C$	11	9.35	1980.07	-3938.10
R4	$1\|H_m + 1\|M_i$	9	39.58	1812.00	-3605.98	R1d	$1\|R_g + 1\|H_m + 1\|R_g{:}M_i + 1\|C$	11	11.57	1978.96	-3935.88
R5	$1\|M_i$	8	55.71	1802.93	-3589.85	R1e	$1\|R_g + 1\|R_g{:}H_m + 1\|M_i + 1\|C$	11	299.15	1835.17	-3648.30
R6	$1\|H_m + 1\|C$	9	455.24	1604.17	-3190.32	R1	$1\|R_g + 1\|H_m + 1\|C + 1\|M_i$	11	301.90	1833.80	-3645.55
R7	1|C	8	461.03	1600.27	-3184.52	R1f	$1\|R_g + 1\|R_g{:}H_m + 1\|M_i + 1\|R_g{:}C$	11	312.44	1828.52	-3635.01
R8	$1\|H_m$	8	470.37	1595.60	-3175.19	R1g	$1\|R_g + 1\|H_m + 1\|M_i + 1\|R_g{:}C$	11	316.41	1826.54	-3631.04
R9	A_i|Fish_ID	7	494.45	1582.56	-3151.10	–	–	–	–	–	–

Table A.3.7. Model selection results for optimal random slopes models. : denotes nested term. LL = log likelihood. K = model complexity. The optimal random effect models are highlighted in bold. A_i = $Age_{increment}$, C = Cohort, H_m = $Hatch_{month}$, M_i = $Month_{increment}$, R_g = Region.

Model #	**Random effects**	**K**	**ΔAIC_c**	**LL**	**AIC_c**
R1a1	**$1\|R_g + A_i\|R_g{:}H_m + A_i\|R_g{:}M_i + 1\|C$**	**15**	**0**	**2188.91**	**-4347.75**
R1a2	$1\|R_g + A_i\|R_g{:}H_m + A_i\|R_g{:}M_i + A_i\|C$	17	1.78	2190.03	-4345.97
R1A.3	$A_i\|R_g + A_i\|R_g{:}H_m + A_i\|R_g{:}M_i + 1\|C$	17	3.42	2189.21	-4344.33
R1a4	$1\|R_g + 1\|R_g{:}H_m + A_i\|R_g{:}M_i + A_i\|C$	15	25.61	2176.10	-4322.14
R1a5	$1\|R_g + 1\|R_g{:}H_m + A_i\|R_g{:}M_i + 1\|C$	13	36.89	2168.45	-4310.86
R1a6	$1\|R_g + A_i\|R_g{:}H_m + 1\|R_g{:}M_i + A_i\|C$	15	289.00	2044.41	-4058.75
R1a7	$1\|R_g + A_i\|R_g{:}H_m + 1\|R_g{:}M_i + 1\|C$	13	289.69	2042.06	-4058.06
R1a8	$1\|R_g + 1\|R_g{:}H_m + 1\|R_g{:}M_i + A_i\|C$	13	390.36	1991.72	-3957.40
R1a	$1\|R_g + 1\|R_g{:}H_m + 1\|R_g{:}M_i + 1\|C$	11	400.30	1984.74	-3947.45

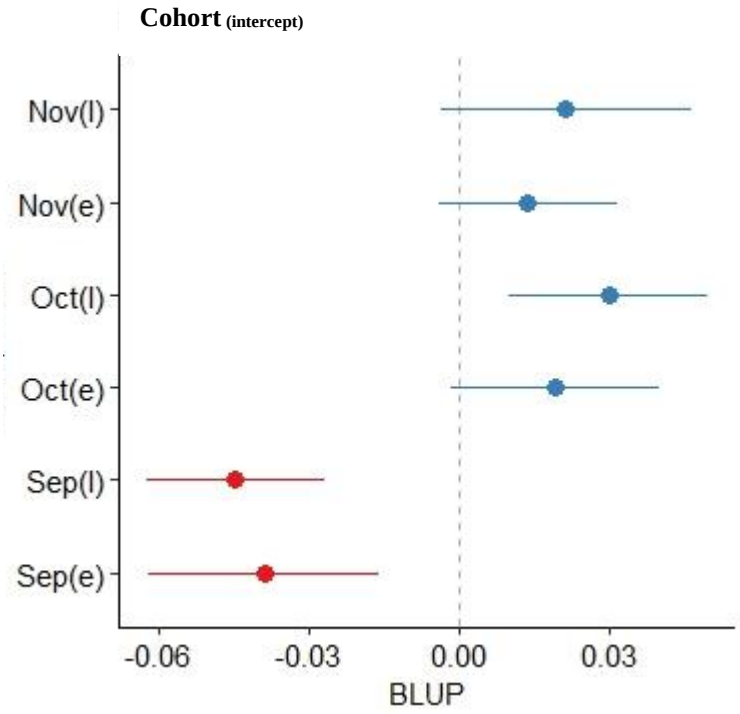


Figure A.3.7. Random intercept plots of average daily growth variation (μmd^{-1}) among cohorts (e = early and l = late) among regions. In each panel the dashed line represents average growth (fixed effect model intercept) among regions Bands are ± SE of the best linear unbiased predictors (BLUPs). Fixed effects fitted are $Age_{increment} + Age_{migration}$.

A.3.2 Supplementary text

A.3.2.1 Linear mixed effects modelling protocol

Linear mixed effects models (LMEM) describing somatic growth (increment width) were fitted according to Morrongiello and Thresher (2015) whereby, intrinsic, random and extrinsic variables are fitted in sequence. All models were initially fitted with fixed effects $Age_{increment}$ and $Age_{migration}$ using maximum likelihood (ML). Random effects structures of increasing complexity (intercepts, nesting and slopes) were then fitted to the intrinsic fixed effects model using restricted estimates of maximum likelihood (REML). Random effects capture variation around average growth and account for the hierarchy inherent in the data (e.g. repeated measurements on individual fish). A random intercept allows for systematic deviations in average growth among the factors of interest (i.e. fish, hatch dates). Random intercepts assume that age-dependent growth increases or decreases in the same manner among all factor levels (Morrongiello and Thresher 2015). To allow for systematic deviations in age-dependent growth trajectories (i.e. shallow or steep), random slopes were modelled.

Once the optimum random effect structure was identified, the intrinsic factor $Age_{migration}$ was removed. This was done to decide if this term should be included in the model (Doubleday et al. 2015). In each instance, $Age_{migration}$ was a significant predictor of growth and was therefore included in all base fixed effects models. The fixed effects component was then optimized followed by the extrinsic component using ML methods.

A.3.2.1.1 Model selection

Competing random, intrinsic and extrinsic models were ranked at each stage and the optimal model describing growth identified through model selection criteria (Burnham 2002). In this case, Akaikes information criterion corrected for small sample sizes was used (AIC_c). The difference between candidate models was calculated (ΔAIC_c) in order to identify the optimal model. Substantial support for a model was found if the ΔAIC_c was > 2 (Burnham 2002). Models with ΔAIC_c less than two have equal explanatory power. In instances where equal support was found, models with the least numbers of parameters (K) were chosen.

A.3.2.1.2 Assumptions and model validation

Otolith increment data was assumed to follow a Gaussian distribution with mean and variance equals to μ and σ^2. Natural log transformation of increment widths and $Age_{migration}$ was done to linearize relationships and meet the assumptions of variance heterogeneity. Collinearity among

predictor variables was explored to avoid multicollinearity. Continuous predictors were mean centered to aid model convergence and the interpretation of random slopes (Morrongiello and Thresher 2015). Centering also allows for the interpretation of main effects independent of the interaction in which terms are involved because the mean for all variables is approximately zero (Schielzeth 2010). Assumptions of statistical tests used were explored and model diagnostics (independence, variance heterogeneity, qqnorm plots and residuals) used to validate model outputs.

Appendix 4

A.4.1 Supplementary data

Table A.4.1. Size parameters and size-based shape indices with calculation formulas used to derive shape indices describing otolith morphology.

Size parameters	**Size-based shape indices**
Area (A)	$\text{Circularity} = \frac{P}{A^2}$
Perimeter (P)	$\text{Rectangularity} = \frac{A}{(O_L \times O_W)}$
Otolith length (O_L)	$\text{Form factor} = \frac{4\pi A}{P^2}$
Otolith width (O_W)	$\text{Roundness} = \frac{4A}{\pi O_L^2}$
	$\text{Ellipticity} = \frac{(O_L - O_W)}{(O_L + O_W)}$
	$\text{Aspect ratio} = \frac{O_L}{O_W}$

Table A.4.2. Results from Kolmogorov-Smirnov tests of differences in length frequency distribution between regions within hatch-seasons. Significant differences are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS denotes non-significant differences.

Hatch-season	**Region**	**D**	**p-value**
Autumn	Golden Bay – Canterbury	0.18	NS
	Buller – Canterbury	0.16	NS
	Golden Bay – Buller	0.26	NS
Winter	Bay of Plenty – Golden Bay	0.77	***
	Bay of Plenty – Buller	0.78	***
	Bay of Plenty – Canterbury	0.52	***
	Golden Bay – Buller	0.44	***
	Golden Bay – Canterbury	0.29	NS
	Buller – Canterbury	0.37	*

Table A.4.3. Spearman's correlation coefficients among shape indices for autumn- and winter-hatched post-larvae. Significant correlations ($p < 0.05$) are shown in **bold.**

	Autumn-hatched					**Winter-hatched**				
Shape index	Roundness	Rectangularity	Form factor	Aspect ratio	Ellipticity	Roundness	Rectangularity	Form factor	Aspect ratio	Ellipticity
Circularity	**0.17**	0.07	**0.15**	**-0.17**	**-0.17**	0.03	0.01	**0.20**	0.01	0.01
Roundness	-	**0.44**	**0.43**	**-0.92**	**-0.92**	-	**0.46**	**0.26**	**-0.83**	**-0.84**
Rectangularity	-	-	**0.24**	-0.08	-0.08	-	-	0.16	0.03	0.03
Form factor	-	-	-	**-0.37**	**-0.37**	-	-	-	**-0.21**	**-0.21**
Aspect ratio	-	-	-	-	**1.00**	-	-	-	-	**1.00**

Table A.4.4. Results from the ANCOVA of the shape indices with otolith length (O_L) as a covariate and region as a factor. b is the common within group slope. NS denotes non-significant differences.

Hatch-season	**Shape indices**	**F**	**p**	**b**
Autumn	$Rectangularity_{(log)}$	0.26	NS	-0.1
	Roundness	0.59	NS	-0.46
	Ellipticity	1.41	NS	0.22
	Aspect ratio	1.46	NS	0.49
Winter	Rectangularity	1.99	NS	–
	Roundness	2.8	NS	-0.17
	Ellipticity	2.04	NS	0.07
	Aspect ratio	2.01	NS	0.17

Table A.4.5. Loading of each of the morphological variables for autumn- and winter-hatched post-larvae from the principal components analysis.

Hatch-season	Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Autumn	A5	-0.38	0.01	0.47	-0.47	0.12	-0.01	0.10	-0.62	0.01
	A8	-0.25	-0.27	-0.46	-0.45	-0.63	0.22	0.01	0.02	0.01
	B6	0.29	0.35	0.34	-0.25	-0.53	-0.55	0.09	0.16	-0.01
	B7	-0.42	-0.08	0.24	0.44	-0.27	0.02	0.70	-0.62	0.01
	C5	-0.42	-0.13	0.25	0.39	-0.27	-0.11	0.68	0.21	-0.01
	C8	-0.29	-0.41	-0.23	-0.16	0.33	-0.71	-0.12	0.18	-0.01
	D3	0.37	-0.33	-0.19	0.35	-0.24	-0.28	0.11	-0.66	0.01
	Aspect ratio	-0.25	0.50	-0.35	0.10	0.01	-0.15	0.05	-0.17	0.71
	Ellipticity	-0.25	0.50	-0.35	0.10	0.01	-0.14	0.04	-0.18	-0.70
Winter	B4	-0.11	0.70	0.71	-0.04	0.01				
	B6	0.06	0.71	-0.69	-0.03	0.01				
	Aspect Ratio	0.58	-0.01	0.07	-0.40	-0.71				
	Roundness	-0.55	-0.06	-0.08	-0.83	0.01				
	Ellipticity	0.58	-0.01	0.07	-0.40	0.71				

Table A.4.6. Stepwise F-to-enter values from the linear discriminant analysis testing for regional differences in otolith morphologies of autumn- and winter-hatched post-larvae among regions.

Hatch-season	**Variable**	**Wilks lambda** (λ)	**F-remove** ($_{8,370}$) **= 4.73**	**p-value**
Autumn	PC1	0.94	13.45	0.001
	PC4	0.84	2.11	0.12
	PC2	0.84	2.07	0.13
	PC5	0.83	1.47	0.23
Winter			**F-remove** ($_{9,353}$) **= 4.62**	
	PC1	0.84	9.15	0.001
	PC2	0.93	3.76	0.01
	PC4	0.97	1.46	0.23

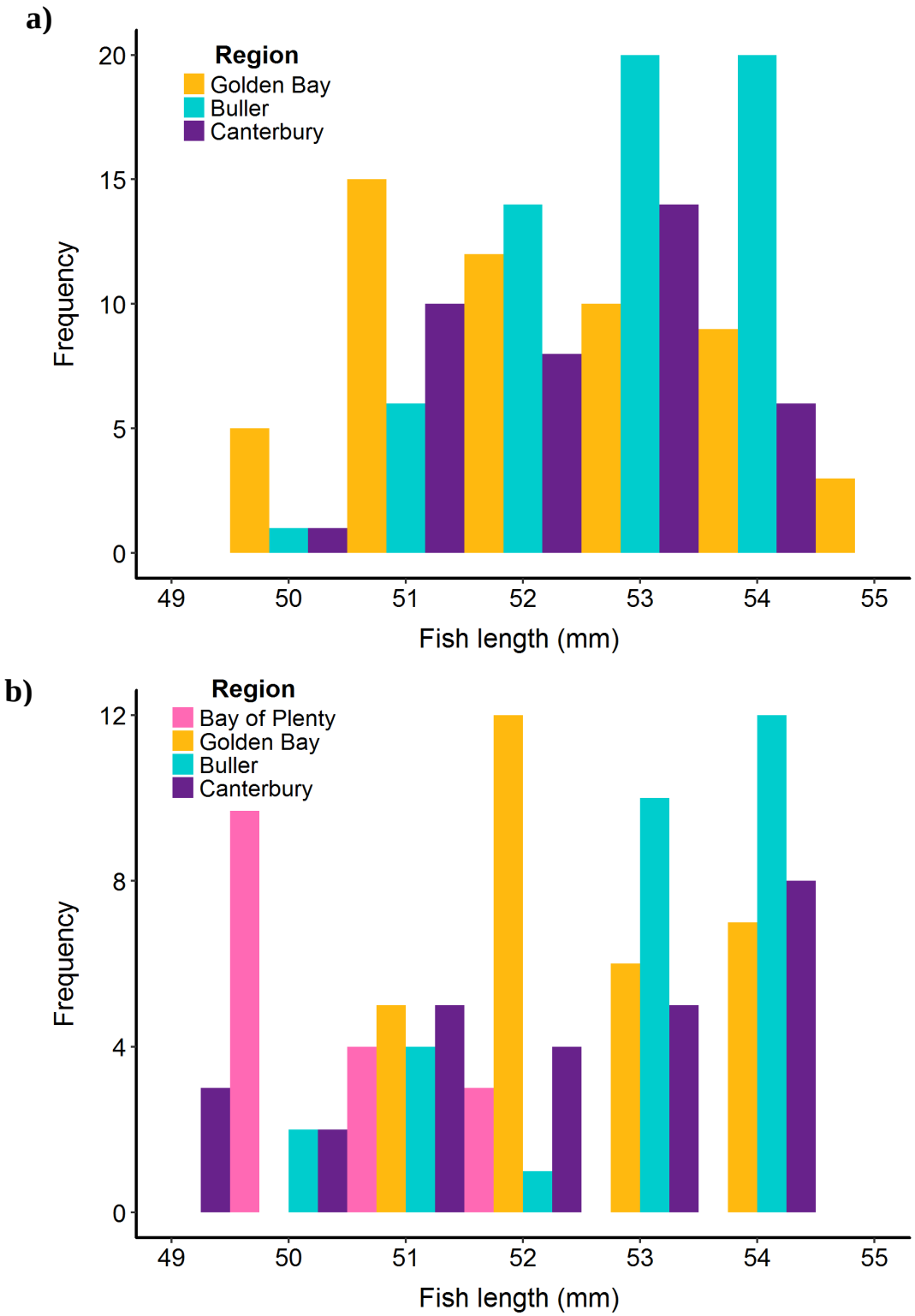


Figure A.4.1. Length-frequency distribution of **a)** autumn- and **b)** winter-hatched post-larvae that were measured for otolith morphology analysis.

Appendix 5

A.5.1 Supplementary data

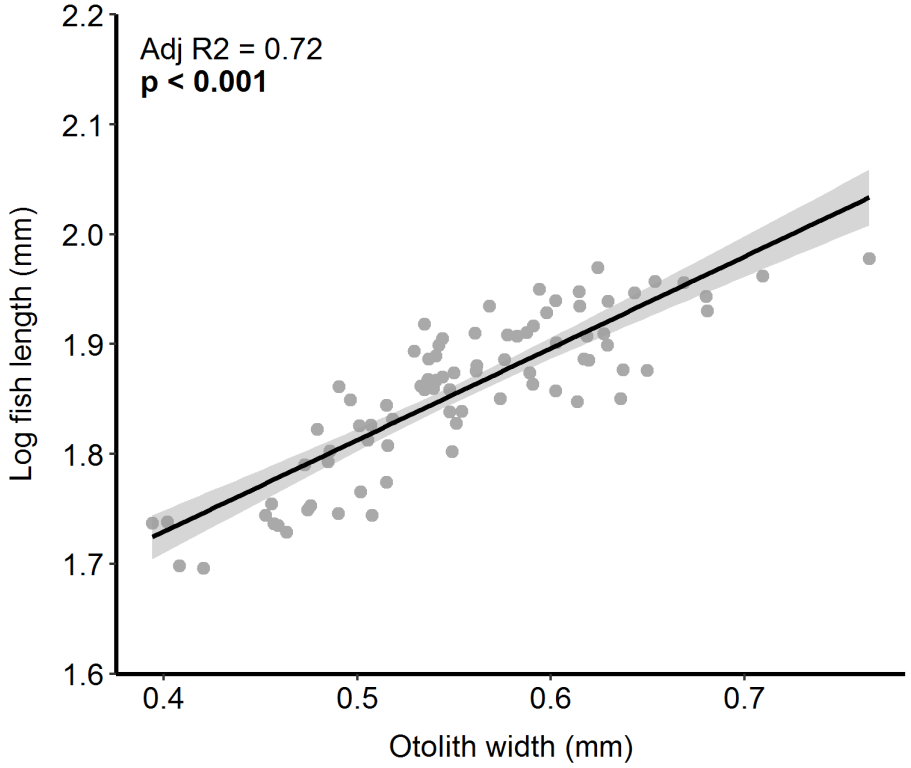


Figure A.5.1. Linear regression showing the relationship between fish-length (mm) and otolith-width (mm) for adult inanga.

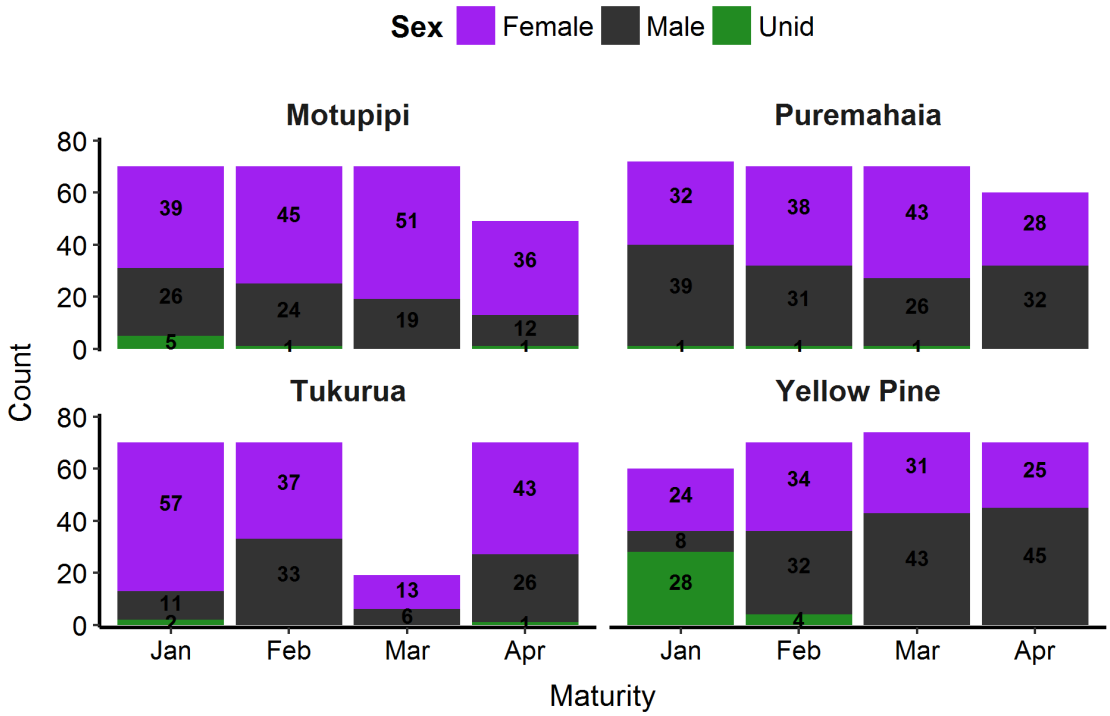


Figure A.5.2. Numbers of adults sampled in each river, month by sex.

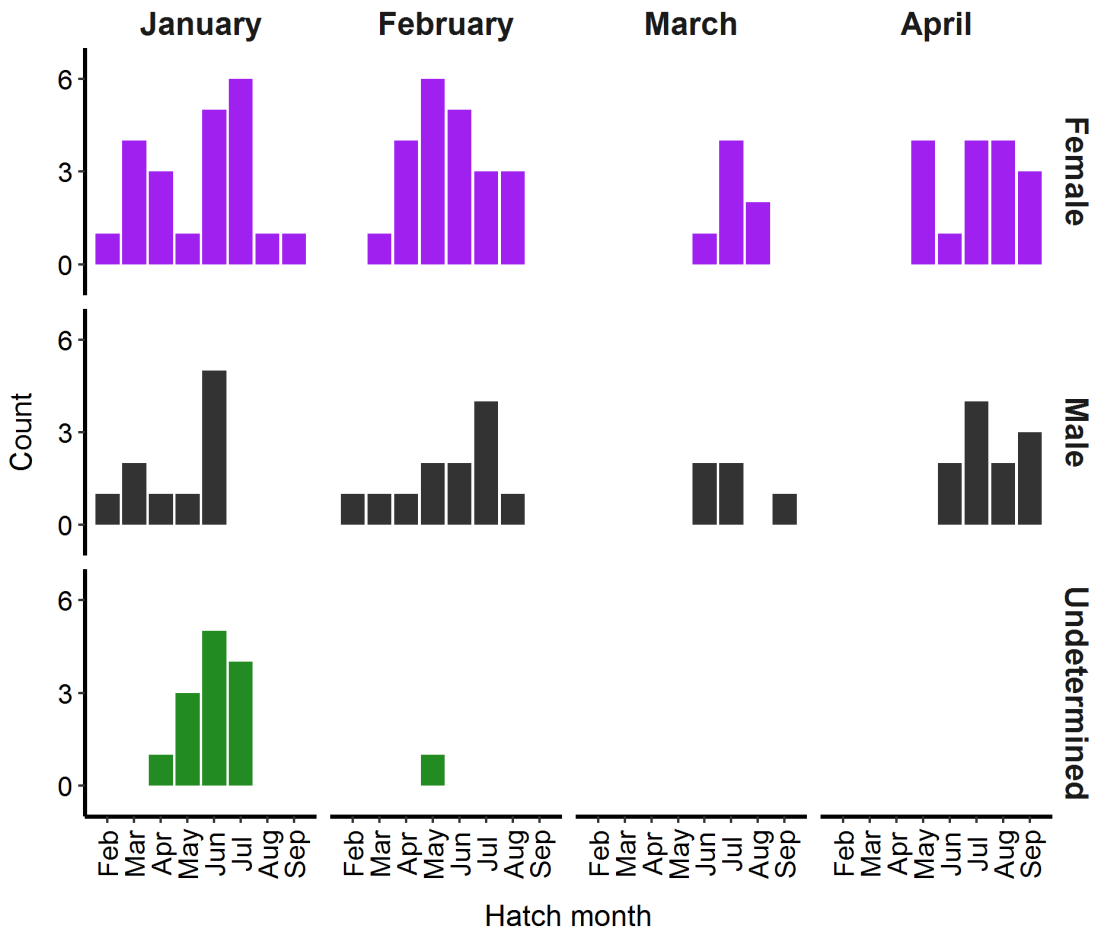


Figure A.5.3. Bar plot showing the numbers of fish in each hatch-month broken down by sex and month at capture.

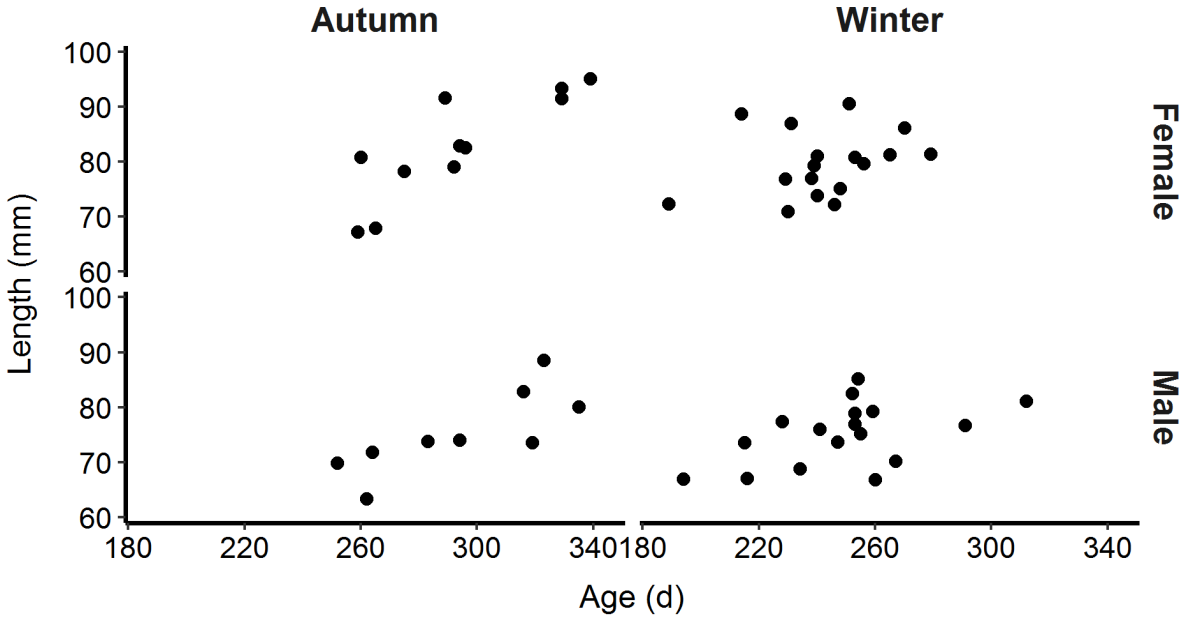


Figure A.5.4. Relationship between size and age at maturity of autumn- and winter-hatched males and females.

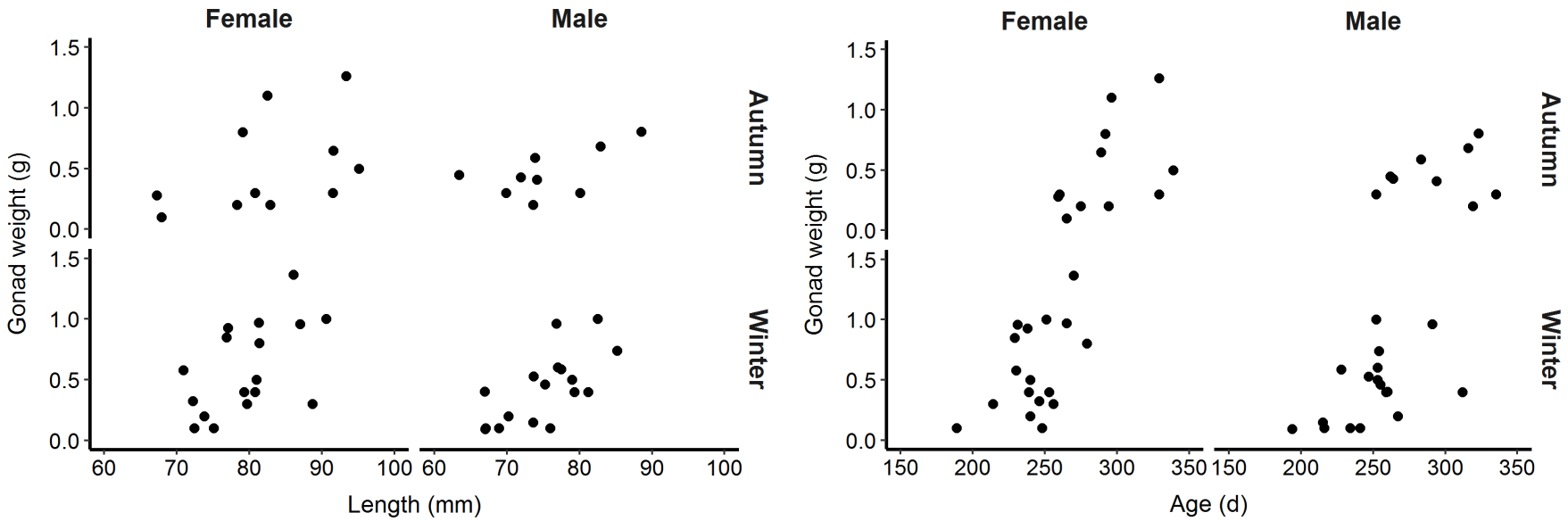


Figure A.5.5. Relationships between gonad weight (g) and length and age at maturity for males and females among hatch-seasons.

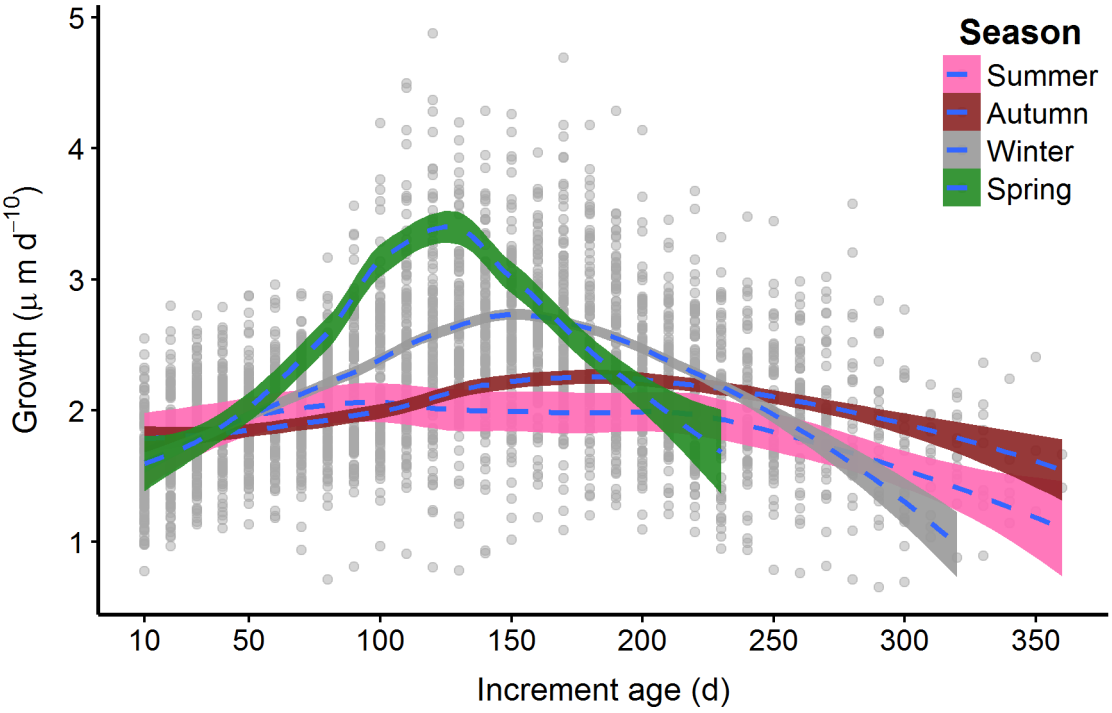


Figure A.5.6. Age-dependent somatic growth trajectory among hatch seasons. A LOESS smother is fitted to visualise non-linear trends in somatic growth. The width of each band is ± 1. S.E of the LOWESS smoother. Summer hatched fish are shown but were not included in the GAMM analysis to model growth among hatch-seasons.

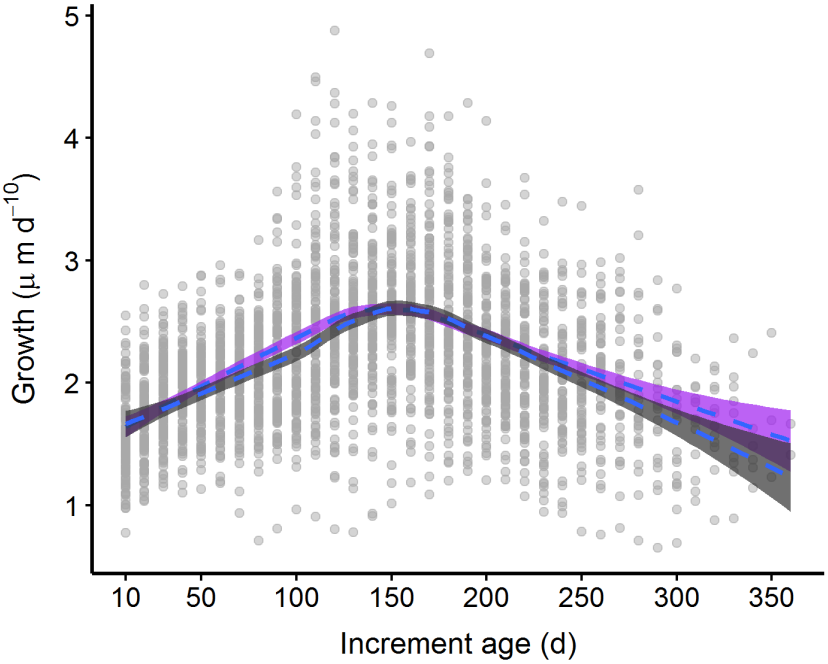


Figure A.5.7.Age-dependent somatic growth trajectory of all males and females sampled. A LOWESS smoother is fitted to visualise non-linear changes in growth with age for each sex (dashed line). The width of the bands is ± 1 S.E of the LOWESS line.

Table A.5.1. Results of ANOVA for the effect of different age-dependent growth trajectories among hatch-seasons on the growth of mature males and mature females. Significance is denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Sex	Hatch-season	F	p
Male	Autumn	5.46	***
	Winter	2.39	**
	Spring	5.79	***
Female	Autumn	3.18	***
	Winter	3.87	***

Mature females

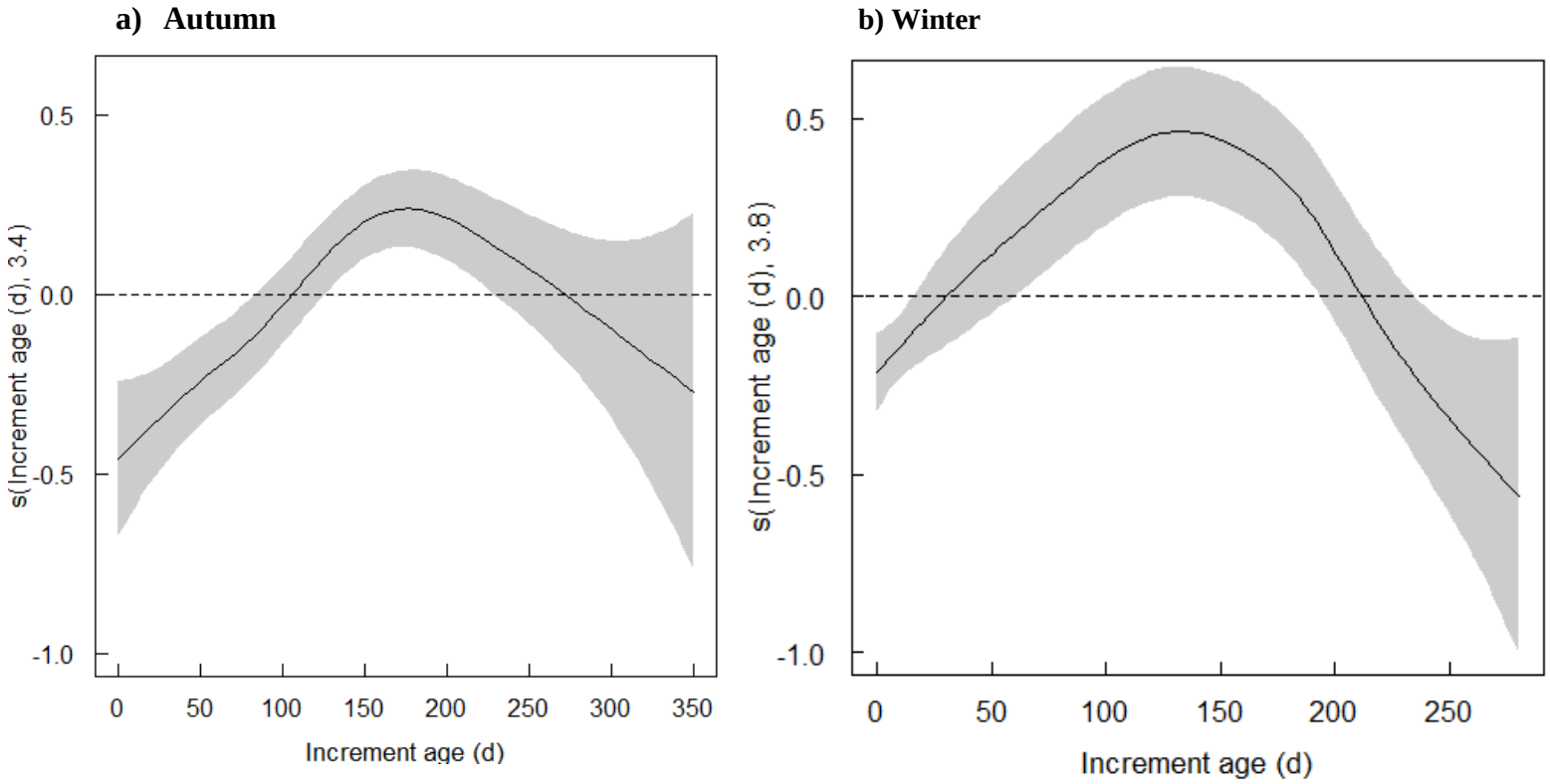


Figure A.5.8. Partial effects plots from the GAMM testing for an interaction between age-dependent growth and hatch-season for mature females. The plots show the predicted smoothers of age-dependent growth rates among hatch seasons for sexually mature females. The estimated degrees of freedom (EDF) are shown on the y-axis. The dashed line is the model intercept (growth of an average fish). The grey shaded lines are the 95% confidence intervals of the predicted smoothers.

Mature males

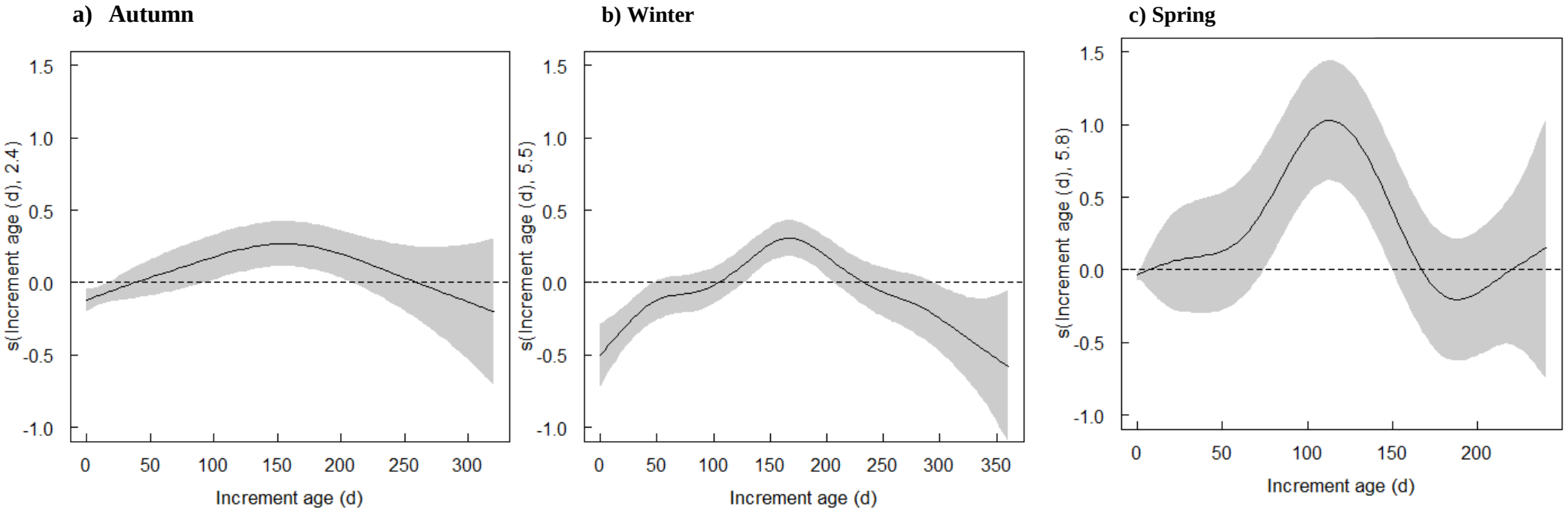


Figure A.5.9. Partial effects plots from the GAMM testing for an interaction between age-dependent growth and hatch-season for mature males. The plots show the predicted smoothers of age-dependent growth among sexually mature males among hatch-seasons. The estimated degrees of freedom (EDF) are shown on the y-axis. The black dashed line is the model intercept and the grey shade lines the 95% confidence intervals of the predicted smoothers.

A.5.2 Supplementary material

A.5.2.1 Data analysis

The effects of sex, maturity (immature/mature) and month at capture (January – April) on fish size (mm) were tested by fitting a three-way interaction (sex*maturity*month) using linear mixed effects models (LMEMs). River was treated as a random effect. This was because rivers were seen as a random sample of all possible rivers in the Golden Bay region and river-specific differences were not of interest for this study. A top-down strategy was used whereby a model containing the maximum interaction possible (in this case a three-way) was fitted initially. Interaction terms were then dropped sequentially and the significance of dropped terms tested using log-likelihood ratio (LR) tests (Zuur 2009). Higher level terms were removed from the model if they were not significant and only significant terms retained. LR tests were used to alleviate issues when testing between factor covariates with greater than 3 levels (river, month) (Zuur 2009). Multiple pairwise comparison tests (Bonferroni corrected) were used to test for significant differences among factor levels where relevant. 95% confidence intervals (CI) of the predicted values are reported.

The numbers of fish in each developmental stages (1-8) were examined across months between males and females to ascertain when peak reproduction occurs.

A.5.2.1.1 Size

Overall, fish length ranged 46.0 mm to 113.2 mm. Average length of males was 73.3 mm (n = 413, SD = 10.5) and females 77.1 mm (n = 576, SD = 11.7, range = 47.4 – 109 mm). Unsexed fish were on average 56.9 mm length (n = 45, SD = 118.13). Nineteen females and 8 males greater than 100 mm were found across the four rivers which collectively comprised 3.7% of all fish sampled. The smallest sexually mature male was 46.0 mm and the largest 109.0 mm (n = 292). For females, the smallest sexually mature fish was 52.8 mm and the largest 111.4 mm (n = 259). Immature males were between 49.9 and 103.9 mm (n = 116) while immature females were 47.4 – 113.27 mm size (n = 308).

The three-way interaction term (sex*maturity*month) fitted was not significant (LR = 5.59, df = 22, $p > 0.1$). The two-way interaction (sex*month) was also not significant (LR = 2.29, df = 19, $p > 0.5$). There was a significant interaction between maturity stage and month at capture on fish size ($F_{3,879} = 38.46$, $p < 0.001$). Mature fish in January were significantly

larger than those in February, March and April otherwise no significant differences were found among mature fish across months (Fig A.5.12, Table A.5.2). Immature fish were mostly similar sizes across the four months except in April when immature fish were significantly larger than those in February (Fig. A.5.12, Table A.5.2). In January mature fish were significantly larger than immature fish ($\beta = 15.32$, CI = 11.16 – 19.48). During February, mature fish were also larger than immature fish ($\beta = 5.10$, CI = 1.93 – 8.27) otherwise no significant differences were found between maturity stages within months (Fig. A.5.12).

The interaction between maturity and sex was significant ($F_{1,879} = 6.02$, $p < 0.05$). Mature females were significantly larger than immature females ($\beta = 4.09$, CI = 2.14 – 6.03) and mature males were significantly smaller than mature females ($\beta = -4.05$, CI = -5.77 – -2.23). No other comparisons were significant.

The inclusion of variance terms showed that the most significant source of variation in size was between maturity stages across months. Both immature and mature fish showed the same pattern in variation with size being the most variable in January and the least in April (Fig. A.5.12). Overall, 13% of the variation was explained by the interactions Sex*Month and Maturity*Month. Conditional on the random effects for each river, 34% variation was explained indicating that differences between rivers are a significant source of variation in size.

Table A.5.2. Multiple comparisons tests with 95% Bonferroni corrected confidence intervals showing significant differences in size among maturity stages and months for all adult inanga sampled.

Maturity	Comparisons	Estimate	95% CI
Mature	February x January	-11.88	-15.74 – -8.02
	March x January	-12.93	-16.47 – -9.40
	April x January	-14.09	-17.84 – -10.33
Immature	April x February	5.23	1.73 – 8.72

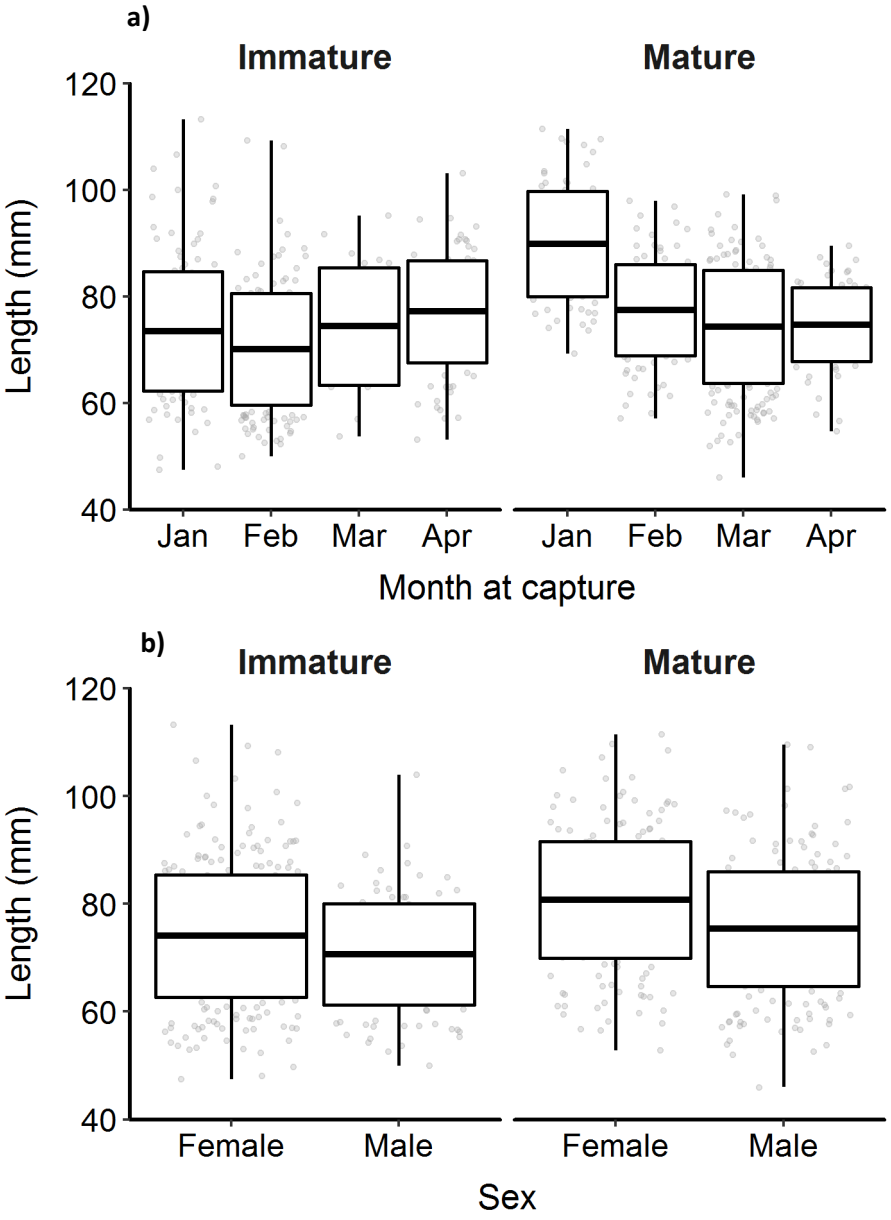


Figure A.5.12. Panel **a)** shows boxplot of mean length (mm) among immature and mature fish in each month. In panel **b)** the boxplot shows mean length (mm) of mature and immature males and females. In each boxplot, lines are the maximum and minimum values observed. The height of the boxes is the within-sample standard deviation.

A.5.2.1.2 Reproductive development

A range of gonad developmental stages were found across months for both sexes. In January and February, there were generally equal numbers of fish with developmental stages 2 through to 6 between sexes (Fig. A.5.13). In March, more mature (greater than stage 5) males and females were found (Fig. A.5.13). In April, females showed a wider range of developmental stages compared to males which were mostly mature or spent. "Recent" (stage 7) spent fish were found in January and February indicating that a spawning event occurred in late summer (Fig. A.5.13). Recently spent fish were also found in April suggesting that these fish survived a previous spawning event (Fig. A.5.13).

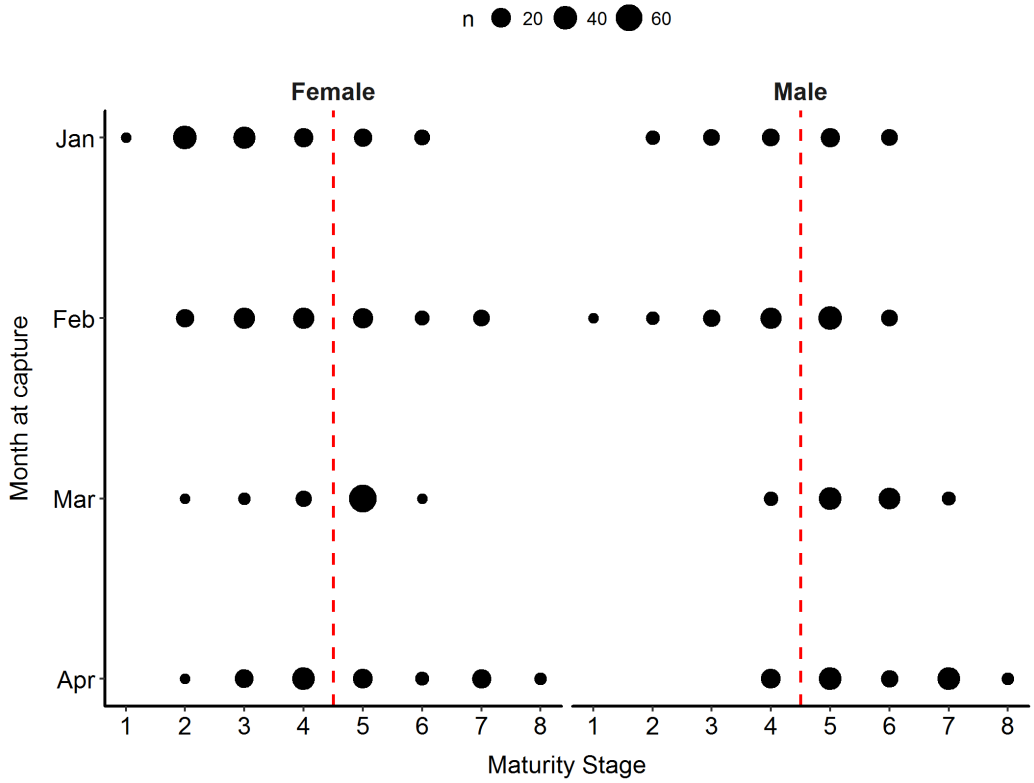


Figure A.5.13. Sexual developmental progression of males and females in each month. The size of the circles denotes the numbers (shown in the legend) of fish in each development category. The dashed red-line separates immature (stages 1-4) from mature (stages 5-6) and spent (stages 7-8) fish.

A.5.2.1.3 Reproductive investment

Spearman's correlations showed that the relationship between length and metrics of reproductive investment varied across months and sex. In January, both mature males and females showed the weakest relationships between gonad weight (M_g) and body size (L_t) (Table A.5.3). The strongest relationships were found in March for both sexes (Table A.5.3). Relationships between size-independent measures of reproductive investment were mostly

non-significant for males and females (Table A.5.3). However, males in February and March showed a significant association between length and I_g.

Table A.5.3. Spearman's correlations showing the relationship between gonad weight (g) and gonado-somatic index (I_g) among male and females that are mature in each month. Significant correlations are denoted by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). NS denotes non-significant correlations.

Metric	**Sex**	**Month**	**r_s**	**p**
Gonad weight (g)	Male	January	0.38	**
		February	0.82	***
		March	0.88	***
		April	0.64	***
	Female	January	0.45	***
		February	0.73	***
		March	0.78	***
		April	0.68	***

Acolas, M. L., J. Labonne, J. L. Baglinière, and J. M. Roussel. 2012. The role of body size versus growth on the decision to migrate: a case study with *Salmo trutta*. Naturwissenschaften **99**:11-21.

Adams, C. E., and F. A. Huntingford. 1997. Growth, maturation and reproductive investment in Arctic charr. Journal of Fish Biology **51**:750-759.

Aguera, A., and D. Brophy. 2011. Use of saggital otolith shape analysis to discriminate Northeast Atlantic and Western Mediterranean stocks of Atlantic saury, *Scomberesox saurus saurus* (Walbaum). Fisheries Research **110**:465-471.

Alemany, F., S. Deudero, B. Morales-Nin, J. L. López-Jurado, J. Jansà, M. Palmer, and I. Palomera. 2006. Influence of physical environmental factors on the composition and horizontal distribution of summer larval fish assemblages off Mallorca island (Balearic archipelago, western Mediterranean). Journal of Plankton Research **28**:473-487.

Ali, M., A. Nicieza, and R. J. Wootton. 2003. Compensatory growth in fishes: a response to growth depression. Fish and Fisheries **4**:147-190.

Allan, B. J. M., P. Domenici, P. L. Munday, and M. I. McCormick. 2015. Feeling the heat: the effect of acute temperature changes on predator-prey interactions in coral reef fish. Conservation Physiology **3**:1-21.

Allibone, R., J. Boubée, and D. West. 1999. The ones that got away: determining whitebait movements and rates of escape. Water & Atmosphere **7**:11-13.

Angilletta, M. J., T. D. Steury, and M. W. Sears. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and Comparative Biology **44**:498-509.

Apte, S., and J. P. A. Gardner. 2002. Population genetic subdivision in the New Zealand greenshell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analysis of mitochondrial DNA. Molecular Ecology **11**:1617-1628.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Quarterly Review of Biology **72**:149-177.

Augspurger, J. M., M. Warburton, and G. P. Closs. 2016. Life-history plasticity in amphidromous and catadromous fishes: a continuum of strategies. Reviews in Fish Biology and Fisheries **27**:1-16.

August, S. M., and B. J. Hicks. 2008. Water temperature and upstream migration of glass eels in New Zealand: implications of climate change. Environmental Biology of Fishes **81**:195-205.

Bailey, K. M., and E. D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Advances in Marine Biology **25**:1-83.

Baker, C. F., and B. J. Hicks. 2003. Attraction of migratory inanga (*Galaxias maculatus*) and koaro (*Galaxias brevipinnis*) juveniles to adult galaxiid odours. New Zealand Journal of Marine and Freshwater Research **37**:291-299.

Barbee, N. C., R. Hale, J. Morrongiello, A. Hicks, D. Semmens, B. J. Downes, and S. E. Swearer. 2011. Large-scale variation in life history traits of the widespread diadromous fish, *Galaxias maculatus*, reflects geographic differences in local environmental conditions. Marine and Freshwater Research **62**:790-800.

Barker, J. R., and D. M. Lambert. 1988. A genetic analysis of populations of *Galaxias maculatus* from the Bay of Plenty: implications for natal river return. New Zealand Journal of Marine and Freshwater Research **22**:321-326.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software. **67**:48.

Baumann, H., G. Tomas, K. Georgs, M. Andrej, F. Valerie, and T. Axel. 2006. Temperature-induced regional and temporal growth differences in Baltic young-of-the-year sprat *Sprattus sprattus*. Marine Ecology Progress Series **317**:225-236.

Begg, G. A., K. D. Friedland, and J. B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research **43**:1-8.

Bell, K. N. I., P. Pepin, and J. A. Brown. 1995. Seasonal inverse cycling of length at recruitment and age at recruitment in the diadromous gobies *Sicydium punctatum* and *Sicydium antillarum* in Dominica, West Indies. Canadian Journal of Fisheries and Aquatic Sciences **52**:1535-1545.

Benzie, V. 1968. Stages in the normal development of *Galaxias maculatus attenuatus* (Jenyns). New Zealand Journal of Marine and Freshwater Research **2**:606-627.

Bergenius, M. A. J., M. I. McCormick, M. G. Meekan, and D. R. Robertson. 2005. Environmental influences on larval duration, growth and magnitude of settlement of a coral reef fish. Marine Biology **147**:291-300.

Bergenius, M. A. J., M. G. Meekan, D. R. Robertson, and M. I. McCormick. 2002. Larval growth predicts the recruitment success of a coral reef fish. Oecologia **131**:521-525.

Bergmann, C. 1847. Uber die Verhaltnisse der Warmeoko-nomie der Thiere zuihrer grosse. Gottinger Studien **1**:595-708.

Berrebi, P., G. Cattaneo-Berrebi, P. Valade, J. F. Ricou, and T. Hoareau. 2005. Genetic homogeneity in eight freshwater populations of *Sicyopterus lagocephalus*, an amphidromous gobiid of La Réunion Island. Marine Biology **148**:179-188.

Bertram, D. F., R. C. Chambers, and W. C. Leggett. 1993. Negative correlations between larval and juvenile growth-rates in Winter flounder - implications of compensatory growth for variation in size-at-age. Marine Ecology Progress Series **96**:209-215.

Bonhomme, V., S. Picq, C. Gaucherel, and J. Claude. 2014. Momocs: outline analysis using R. **56**:24.

Boy, C. C., A. F. Perez, D. A. Fernandez, J. Calvo, and E. R. Morriconi. 2009a. Energy allocation in relation to spawning and overwintering of a diadromous Puyen (*Galaxias maculatus*) population in the southernmost limit of the species distribution. Polar Biology **32**:9-14.

Boy, C. C., A. F. Pérez, M. E. Lattuca, J. Calvo, and E. Morriconi. 2009b. Reproductive biology of *Galaxias maculatus* (Jenyns 1842) in the Río Ovando, a high-latitude environment in southernmost Patagonia, Argentina. Journal of Applied Ichthyology **25**:661-668.

Bradbury, I. R., B. Laurel, P. V. R. Snelgrove, P. Bentzen, and S. E. Campana. 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proceedings of the Royal Society B: Biological Sciences **275**:1803-1809.

Bradford, J. M., P. P. Lapennas, R. A. Murtagh, F. H. Chang, and V. Wilkinson. 1986. Factors controlling summer phytoplankton production in greater Cook Strait, New Zealand. New Zealand Journal of Marine and Freshwater Research **20**:253-279.

Braun, D. C., and J. D. Reynolds. 2014. Life history and environmental influences on population dynamics in sockeye salmon. Canadian Journal of Fisheries and Aquatic Sciences **71**:1198-1208.

Brophy, D., and B. S. Danilowicz. 2002. Tracing populations of Atlantic herring (*Clupea harengus* L.) in the Irish and Celtic Seas using otolith microstructure. ICES Journal of Marine Science **59**:1305-1313.

Brophy, D., and B. S. Danilowicz. 2003. The influence of pre-recruitment growth on subsequent growth and age at first spawning in Atlantic herring (*Clupea harengus* L.). ICES Journal of Marine Science **60**:1103-1113.

Brophy, D., P. Haynes, H. Arrizabalaga, I. Fraile, J. M. Fromentin, F. Garibaldi, I. Katavic, F. Tinti, F. S. Karakulak, D. Macias, D. Busawon, A. Hanke, A. Kimoto, O. Sakai, S. Deguara, N. Abid, and M. N. Santos. 2016. Otolith shape variation provides a marker of stock origin for north Atlantic bluefin tuna (*Thunnus thynnus*). Marine and Freshwater Research **67**:1023-1036.

Brophy, D., and P. A. King. 2007. Larval otolith growth histories show evidence of stock structure in Northeast Atlantic blue whiting (*Micromesistius poutassou*). ICES Journal of Marine Science **64**:1136-1144.

Brown, J. S., G. H. Darcy, and W. Overholtz. 1987. Stock assessment/stock identification: an interactive process. NOAA Tech. Memo. NMFS-SEFSC 199, 1-19.

Bruce, B. D., K. Evans, C. A. Sutton, J. W. Young, and D. M. Furlani. 2001. Influence of mesoscale oceanographic processes on larval distribution and stock structure in jackass morwong (*Nemadactylus macropterus* : Cheilodactylidae). ICES Journal of Marine Science **58**:1072-1080.

Burke, N., D. Brophy, and P. A. King. 2008. Otolith shape analysis: its application for discriminating between stocks of Irish Sea and Celtic Sea herring (*Clupea harengus*) in the Irish Sea. ICES Journal of Marine Science **65**:1670-1675.

Burnet, A. M. R. 1965. Observations on the spawning migrations of *Galaxias attenuatus*. New Zealand Journal of Science **8**:79-87.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical Information-Theoretic approach. 2nd edition. Springer, Berlin.

Cadrin, S. X. 2000. Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries **10**:91-112.

Campana, S. E., and J. M. Casselman. 1993. Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences **50**:1062-1083.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences **42**:1014-1032.

Cardinale, M., P. Doering-Arjes, M. Kastowsky, and H. Mosegaard. 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences **61**:158-167.

Carlson, S. M., A. P. Hendry, and B. H. Letcher. 2004. Natural selection acting on body size, growth rate and compensatory growth: an empirical test in a wild trout population. Evolutionary Ecology Research **6**:955-973.

Carrea, C., V. E. Cussac, and D. E. Ruzzante. 2013. Genetic and phenotypic variation among *Galaxias maculatus* populations reflects contrasting landscape effects between northern and southern Patagonia. Freshwater Biology **58**:36-49.

Chang, F. H., J. Zeldis, M. Gall, and J. Hall. 2003. Seasonal and spatial variation of phytoplankton assemblages, biomass and cell size from spring to summer across the north-eastern New Zealand continental shelf. Journal of Plankton Research **25**:737-758.

Chapman, A., D. L. Morgan, S. J. Beatty, and H. S. Gill. 2006. Variation in life history of land-locked lacustrine and riverine populations of *Galaxias maculatus* (Jenyns 1842) in Western Australia. Environmental Biology of Fishes **77**:21-37.

Chapman, B. B., C. Bronmark, J. A. Nilsson, and L. A. Hansson. 2011. The ecology and evolution of partial migration. Oikos **120**:1764-1775.

Chiswell, S. M., H. C. Bostock, P. J. H. Sutton, and M. J. M. Williams. 2015. Physical oceanography of the deep seas around New Zealand: a review. New Zealand Journal of Marine and Freshwater Research **49**:286-317.

Chiswell, S. M., and G. J. Rickard. 2011. Larval connectivity of harbours via ocean currents: a New Zealand study. Continental Shelf Research **31**:1057-1074.

Chubb, A. L., R. M. Zink, and J. M. Fitzsimons. 1998. Patterns of mtDNA variation in Hawaiian freshwater fishes: the phylogeographic consequences of amphidromy. Journal of Heredity **89**:8-16.

Ciannelli, L., and K. M. Bailey. 2005. Landscape dynamics and resulting species interactions: the cod-capelin system in the southeastern Bering Sea. Marine Ecology Progress Series **291**:227-236.

Ciotti, B. J., T. E. Targett, R. D. M. Nash, and A. J. Geffen. 2014. Growth dynamics of European plaice *Pleuronectes platessa* L. in nursery areas: a review. Journal of Sea Research **90**:64-82.

Clobert, J., J. F. Le Galliard, J. Cote, S. Meylan, and M. Massot. 2009. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. Ecology Letters **12**:197-209.

Closs, G. P., A. S. Hicks, and P. G. Jellyman. 2013. Life histories of closely related amphidromous and non-migratory fish species: a trade-off between egg size and fecundity. Freshwater Biology **58**:1162-1177.

Closs, G. P., and M. Warburton. 2016. Life histories of amphidromous fishes. Pages 102-122 *in* D. F. Morais P, editor. An introduction to fish migration. CRC Press, Boca Raton.

Conover, D. O. 1992. Seasonality and the scheduling of life-history at different latitudes. Journal of Fish Biology **41**:161-178.

Conroy, C. W., P. M. Piccoli, and D. H. Secor. 2015. Carryover effects of early growth and river flow on partial migration in striped bass *Morone saxatilis*. Marine Ecology Progress Series **541**:179-194.

Correia, A. T., C. Antunes, E. J. Isidro, and J. Coimbra. 2003. Changes in otolith microstructure and microchemistry during larval development of the European conger eel (*Conger conger*). Marine Biology **142**:777-789.

Cowen, R. K. 1991. Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus pulcher*. Marine Ecology Progress Series **69**:9-15.

Crean, A. J., and D. J. Marshall. 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. Philosophical Transactions of the Royal Society B: Biological Sciences **364**:1087-1096.

Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Pages 249-293 *in* J. H. S. Blaxter and A. J. Southward, editors. Advances in Marine Biology. Academic Press.

David, B., L. Chadderton, G.P. Closs, B. Barry, A. Markwitz. 2004. Evidence of flexible recruitment strategies in coastal populations of giant kokopu (*Galaxias argenteus*). Department of Conservation, Wellington, New Zealand.

Dingle, H., and V. A. Drake. 2007. What is migration? BioScience **57**:113-121.

Doubleday, Z. A., C. Izzo, J. A. Haddy, J. M. Lyle, Q. Ye, and B. M. Gillanders. 2015. Long-term patterns in estuarine fish growth across two climatically divergent regions. Oecologia **179**:1079-1090.

Duarte-Neto, P., R. Lessa, B. Stosic, and E. Morize. 2008. The use of sagittal otoliths in discriminating stocks of common dolphinfish (*Coryphaena hippurus*) off northeastern Brazil using multishape descriptors. ICES Journal of Marine Science **65**:1144-1152.

Duarte, C. M., and M. Alcaraz. 1989. To produce many small or few large eggs: a size-independent reproductive tactic of fish. Oecologia **80**:401-404.

Feutry, P., A. Vergnes, D. Broderick, J. Lambourdiere, P. Keith, and J. R. Ovenden. 2013. Stretched to the limit; can a short pelagic larval duration connect adult populations of an Indo-Pacific diadromous fish (*Kuhlia rupestris*)? Molecular Ecology **22**:1518-1530.

Fiksen, O., C. Jorgensen, T. Kristiansen, F. Vikebo, and G. Huse. 2007. Linking behavioural ecology and oceanography: larval behaviour determines growth, mortality and dispersal. Marine Ecology Progress Series **347**:195-205.

Fitzhugh, G. R., S. W. Nixon, D. W. Ahrenholz, and J. A. Rice. 1997. Temperature effects on otolith microstructure and birth month estimation from otolith increment patterns in Atlantic menhaden. Transactions of the American Fisheries Society **126**:579-593.

Folkvord, A., G. Gundersen, J. Albretsen, L. Asplin, S. Kaartvedt, and J. Giske. 2016. Impact of hatch date on early life growth and survival of Mueller's pearlside (*Maurolicus muelleri*) larvae and life-history consequences. Canadian Journal of Fisheries and Aquatic Sciences **73**:163-176.

Fortier, L., and W. C. Leggett. 1983. Vertical migrations and transport of larval fish in a partially mixed estuary. Canadian Journal of Fisheries and Aquatic Sciences **40**:1543-1555.

Foster, B. A., and W. R. Battaerd. 1985. Distribution of zooplankton in a coastal upwelling in New Zealand. New Zealand Journal of Marine and Freshwater Research **19**:213-226.

Fowler, A. J., K. P. Black, and G. P. Jenkins. 2000. Determination of spawning areas and larval advection pathways for King George whiting in southeastern Australia using otolith microstructure and hydrodynamic modelling. II. South Australia. Marine Ecology Progress Series **199**:243-254.

Frederiksen, M., J. D. Lebreton, R. Pradel, R. Choquet, and O. Gimenez. 2014. Identifying links between vital rates and environment: a toolbox for the applied ecologist. Journal of Applied Ecology **51**:71-81.

Freshwater, C., M. Trudel, T. D. Beacham, L. Godbout, C.-E. M. Neville, S. Tucker, and F. Juanes. 2015. Disentangling individual- and population-scale processes within a latitudinal size gradient in sockeye salmon. Canadian Journal of Fisheries and Aquatic Sciences **73**:1190-1201.

Freshwater, C., M. Trudel, T. D. Beacham, L. Godbout, C.-E. M. Neville, S. Tucker, and F. Juanes. 2016. Divergent migratory behaviours associated with body size and ocean entry phenology in juvenile sockeye salmon. Canadian Journal of Fisheries and Aquatic Sciences **73**:1723-1732.

Friedland, K. D., R. E. Haas, and T. F. Sheehan. 1996. Post-smolt growth, maturation, and survival of two stocks of Atlantic salmon. Fishery Bulletin **94**:654-663.

Gagliano, M., and M. I. McCormick. 2004. Feeding history influences otolith shape in tropical fish. Marine Ecology Progress Series **278**:291-296.

Gagliano, M., M. I. McCormick, and M. G. Meekan. 2007. Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. Proceedings of the Royal Society B: Biological Sciences **274**:1575-1582.

Gahagan, B. I., K. E. Gherard, and E. T. Schultz. 2010. Environmental and endogenous factors influencing emigration in juvenile anadromous alewives. Transactions of the American Fisheries Society **139**:1069-1082.

Galley, E. A., P. J. Wright, and F. M. Gibb. 2006. Combined methods of otolith shape analysis improve identification of spawning areas of Atlantic cod. ICES Journal of Marine Science **63**:1710-1717.

Garrido, S., R. Ben-Hamadou, A. M. P. Santos, S. Ferreira, M. A. Teodósio, U. Cotano, X. Irigoien, M. A. Peck, E. Saiz, and P. Ré. 2015. Born small, die young: Intrinsic, size-selective mortality in marine larval fish. Scientific Reports **5**:1-10.

Garvey, J. E., T. P. Herra, and W. C. Leggett. 2002. Protracted reproduction in sunfish: the temporal dimension in fish recruitment revisited. Ecological Applications **12**:194-205.

Gillanders, B. M., B. A. Black, M. G. Meekan, and M. A. Morrison. 2012. Climatic effects on the growth of a temperate reef fish from the Southern Hemisphere: a biochronological approach. Marine Biology **159**:1327-1333.

Gillespie, P. A., R. W. Forrest, B. R. Knight, C. D. Cornelisen, and R. G. Young. 2011. Variation in nutrient loading from the Motueka River into Tasman Bay, New Zealand, 2005-2009: implications for the river plume ecosystem. New Zealand Journal of Marine and Freshwater Research **45**:497-512.

Goodman, J. M., N. R. Dunn, P. J. Ravenscroft, R. M. Allibone, J. A. T. Boubée, B. O. David, M. Griffiths, N. Ling, R. A. Hitchmough, and J. Rolfe. 2014. Conservation status of New Zealand freshwater fish, 2013. Department of Conservation, Wellington, New Zealand

Górski, K., E. M. Habit, M. A. Pingram, and A. J. Manosalva. 2015. Variation of the use of marine resources by *Galaxias maculatus* in large Chilean rivers. Hydrobiologia:1-13.

Goto, A., H. Arioka, and R. Yokoyama. 2014. Plastic life-history variation along the course of a steep mountainous river in male *Cottus hangiongensis* (Pisces: Cottidae). Environmental Biology of Fishes **97**:909-919.

Graham, D. H. 1956. A treasury of New Zealand fishes. Reed, Wellington, New Zealand.

Gregory, S. D., M. Nevoux, W. D. Riley, W. R. C. Beaumont, N. Jeannot, R. B. Lauridsen, F. Marchand, L. J. Scott, and J.-M. Roussel. 2017. Patterns on a parr: Drivers of long-term salmon parr length in U.K. and French rivers depend on geographical scale. Freshwater Biology:1-13.

Grimes, C. B., and M. J. Kingsford. 1996. How do riverine plumes of different sizes influence fish larvae: Do they enhance recruitment? Marine and Freshwater Research **47**:191-208.

Gross, M. R. 1987. Evolution of diadromy in fishes. American Fisheries Society Symposium **1**:14-25.

Gross, M. R., R. M. Coleman, and R. M. McDowall. 1988. Aquatic productivity and the evolution of diadromous fish migration. Science **239**:1291-1293.

Ha, P. Y., and R. A. Kinzie. 1996. Reproductive biology of *Awaous guamensis*, an amphidromous Hawaiian goby. Environmental Biology of Fishes **45**:383-396.

Hadfield, M. G., and J. Sharples. 1996. Modelling mixed layer depth and plankton biomass off the west coast of South Island, New Zealand. Journal of Marine Systems **8**:1-29.

Hale, R., and S. E. Swearer. 2008. Otolith microstructural and microchemical changes associated with settlement in the diadromous fish *Galaxias maculatus*. Marine Ecology Progress Series **354**:229-234.

Hale, R., S. E. Swearer, and B. J. Downes. 2009. Is settlement at small spatial scales by diadromous fishes from the Family Galaxiidae passive or active in a small coastal river? Marine and Freshwater Research **60**:971-975.

Halttunen, E., J. L. A. Jensen, T. F. Naesje, J. G. Davidsen, E. B. Thorstad, C. M. Chittenden, S. Hamel, R. Primicerio, and A. H. Rikardsen. 2013. State-dependent migratory timing of postspawned Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences **70**:1063-1071.

Hamer, P. A., J. Kemp, S. Robertson, and J. S. Hindell. 2012. Multiple otolith techniques aid stock discrimination of a broadly distributed deepwater fishery species, blue grenadier, *Macruronus novaezelandiae*. Fisheries Research **113**:21-34.

Hamilton, S. 2008. Larval history influences post-metamorphic condition in a coral-reef fish. Oecologia **158**:449-461.

Hare, J. A., and R. K. Cowen. 1997. Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology **78**:2415-2431.

Heath, R. A. 1978. Semi-diurnal tides in Cook Strait. New Zealand Journal of Marine and Freshwater Research **12**:87-97.

Heath, R. A. 1985. A review of the physical oceanography of the seas around New Zealand - 1982. New Zealand Journal of Marine and Freshwater Research **19**:79-124.

Heim, K. C., M. S. Wipfli, M. S. Whitman, and A. C. Seitz. 2016. Body size and condition influence migration timing of juvenile Arctic grayling. Ecology of Freshwater Fish **25**:156-166.

Hickford, M. J. H., and D. R. Schiel. 2003. Comparative dispersal of larvae from demersal versus pelagic spawning fishes. Marine Ecology Progress Series **252**:255-271.

Hickford, M. J. H., and D. R. Schiel. 2011a. Population sinks resulting from degraded habitats of an obligate life-history pathway. Oecologia **166**:131-140.

Hickford, M. J. H., and D. R. Schiel. 2011b. Synergistic interactions within disturbed habitats between temperature, relative humidity and UVB radiation on egg survival in a diadromous fish. Plos One **6**:e24318.

Hickford, M. J. H., and D. R. Schiel. 2016. Otolith microchemistry of the amphidromous *Galaxias maculatus* shows recruitment to coastal rivers from unstructured larval pools. Marine Ecology Progress Series **548**:197-207.

Hicks, A., B. Leigh, and J. Dare. 2013. Potential inanga spawning areas in Southland Rivers. 2014-06, Environment Southland, Invercargill.

Hicks, A. S., G. P. Closs, and S. E. Swearer. 2010. Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: A multi-element approach for tracking diadromous migrations. Journal of Experimental Marine Biology and Ecology **394**:86-97.

Hicks, B. J., D. W. West, B. J. Barry, A. Markwitz, C. F. Baker, and C. P. Mitchell. 2005. Chronosequences of strontium in the otoliths of two New Zealand migratory freshwater fish, inanga (*Galaxias maculatus*) and koaro (*G. brevepinnis*). International Journal of PIXE **15**:95-101.

Hill, J. 2013. Reproductive biology, movement and spawning dynamics of *Galaxias maculatus* in central New Zealand. MSc., University of Canterbury, Christchurch, New Zealand.

Hinrichsen, H. H., R. Voss, B. Huwer, and C. Clemmesen. 2010. Variability of larval Baltic sprat (*Sprattus sprattus* L.) otolith growth: a modeling approach combining spatially and temporally resolved biotic and abiotic environmental key variables. Fisheries Oceanography **19**:463-479.

Hoareau, T. B., R. Lecomte-Finiger, H. P. Grondin, C. Conand, and P. Berrebi. 2007. Oceanic larval life of La Réunion 'bichiques', amphidromous gobiid post-larvae. Marine Ecology Progress Series **333**:303-308.

Hogan, F., and K. D. Friedland. 2010. Retrospective growth analysis of Atlantic salmon *Salmo salar* and implications for abundance trends. Journal of Fish Biology **76**:2502-2520.

Hogan, J. D., M. J. Blum, J. F. Gilliam, N. Bickford, and P. B. McIntyre. 2014. Consequences of alternative dispersal strategies in a putatively amphidromous fish. Ecology **95**:2397-2408.

Hogan, J. D., R. Kozdon, M. J. Blum, J. F. Gilliam, J. W. Valley, and P. B. McIntyre. 2017. Reconstructing larval growth and habitat use in an amphidromous goby using otolith increments and microchemistry. Journal of Fish Biology **90**:1338-1355.

Houde, E. D. 1989. Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin **87**:471-495.

Huey, J. A., D. A. Crook, J. I. Macdonald, D. J. Schmidt, J. C. Marshall, S. R. Balcombe, R. J. Woods, and J. M. Hughes. 2014. Is variable connectivity among populations of a

continental gobiid fish driven by local adaptation or passive dispersal? Freshwater Biology **59**:1672-1686.

Hughes, J. M., D. J. Schmidt, J. I. Macdonald, J. A. Huey, and D. A. Crook. 2014. Low interbasin connectivity in a facultatively diadromous fish: evidence from genetics and otolith chemistry. Molecular Ecology **23**:1000-1013.

Hüssy, K. 2008. Otolith shape in juvenile cod (*Gadus morhua*): ontogenetic and environmental effects. Journal of Experimental Marine Biology and Ecology **364**:35-41.

Iafrate, J., and K. Oliveira. 2008. Factors affecting migration patterns of juvenile river herring in a coastal Massachusetts stream. Environmental Biology of Fishes **81**:101-110.

Iguchi, K. 2007. Limitations of early seaward migration success in amphidromous fishes. Bishop Museum Bulletin in Cultural and Environmental Studies **3**:75-80.

Iguchi, K., M. Konishi, and H. Takeshima. 2006. Early dispersal of ayu during marine stages as inferred from geographic variation in the number of vertebrae. Fisheries Science **72**:737-741.

Iida, M., S. Watanabe, A. Shinoda, and K. Tsukamoto. 2008. Recruitment of the amphidromous goby *Sicyopterus japonicus* to the estuary of the Ota River, Wakayama, Japan. Environmental Biology of Fishes **83**:331-341.

Iida, M., S. Watanabe, and K. Tsukamoto. 2015. Oceanic larval duration and recruitment mechanism of the amphidromous fish *Sicyopterus japonicus* (Gobioidei: Sicydiinae). Regional Studies in Marine Science **1**:25-33.

Jarvis, M. G., and G. P. Closs. 2015. Larval drift of amphidromous *Gobiomorphus* spp. in a New Zealand coastal stream: a critical spatial and temporal window for protection. New Zealand Journal of Marine and Freshwater Research **49**:439-447.

Johnson, D. W., K. Grorud-Colvert, S. Sponaugle, and B. X. Semmens. 2014. Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecology Letters **17**:743-755.

Jónsdóttir, I. G., S. E. Campana, and G. Marteinsdottir. 2006. Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.). ICES Journal of Marine Science **63**:1501-1512.

Jonsson, B., and N. Jonsson. 2014. Early environment influences later performance in fishes. Journal of Fish Biology **85**:151-188.

Jowett, I. G., and M. J. Duncan. 1990. Flow variability in New Zealand rivers and its relationship to in-stream habitat and biota. New Zealand Journal of Marine and Freshwater Research **24**:305-317.

Keating, J. P., D. Brophy, R. A. Officer, and E. Mullins. 2014. Otolith shape analysis of blue whiting suggests a complex stock structure at their spawning grounds in the Northeast Atlantic. Fisheries Research **157**:1-6.

Keith, P. 2003. Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and the Caribbean regions. Journal of Fish Biology **63**:831-847.

Keith, P., T. B. Hoareau, C. Lord, O. Ah-Yane, G. Gimonneau, T. Robinet, and P. Valade. 2008. Characterisation of post-larval to juvenile stages, metamorphosis and recruitment of an amphidromous goby, *Sicyopterus lagocephalus* (Pallas) (Teleostei : Gobiidae : Sicydiinae). Marine and Freshwater Research **59**:876-889.

Kendall, M. S., M. Poti, T. T. Wynne, B. P. Kinlan, and L. B. Bauer. 2013. Consequences of the life history traits of pelagic larvae on interisland connectivity during a changing climate. Marine Ecology Progress Series **489**:43-59.

Kerr, L. A., and D. H. Secor. 2010. Latent effects of early life history on partial migration for an estuarine-dependent fish. Environmental Biology of Fishes **89**:479-492.

Koster, W. M., D. R. Dawson, and D. A. Crook. 2013. Downstream spawning migration by the amphidromous Australian grayling (*Prototroctes maraena*) in a coastal river in south-eastern Australia. Marine and Freshwater Research **64**:31-41.

Kuhl, F. P., and C. R. Giardina. 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing **18**:236-258.

Lagarde, R., N. Teichert, H. Grondin, H. Magalon, A. Pirog, and D. Ponton. 2016. Temporal variability of larval drift of tropical amphidromous gobies along a watershed in Réunion Island. Canadian Journal of Fisheries and Aquatic Sciences:1-10.

Laurenson, L. J. B., R. P. French, P. Jones, D. Ierodiaconou, S. Gray, V. L. Versace, A. Rattray, S. Brown, and J. Monk. 2012. Aspects of the biology of *Galaxias maculatus*. Journal of Fish Biology **81**:1085-1100.

Leis, J. M. 2007. Behaviour as input for modelling dispersal of fish larvae: behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. Marine Ecology Progress Series **347**:185-193.

Lejeune, L., H. Tabouret, L. Taillebois, D. Monti, and P. Keith. 2016. Larval traits of the Caribbean amphidromous goby *Sicydium punctatum* (Gobioidei: Sicydiinae) in Guadeloupe. Ecology of Freshwater Fish **25**:272-280.

Lester, S. E., and B. I. Ruttenberg. 2005. The relationship between pelagic larval duration and range size in tropical reef fishes: a synthetic analysis. Proceedings of the Royal Society B: Biological Sciences **272**:585-591.

Lewis, B., W. S. Grant, R. E. Brenner, and T. Hamazaki. 2015. Changes in size and age of Chinook Salmon *Oncorhynchus tshawytscha* returning to Alaska. Plos One **10**:e0132872.

Libungan, L. A., G. J. Oskarsson, A. Slotte, J. A. Jacobsen, and S. Palsson. 2015. Otolith shape: a population marker for Atlantic herring *Clupea harengus*. Journal of Fish Biology **86**:1377-1395.

Limburg, K. E. 2001. Through the gauntlet again: demographic restructuring of American shad by migration. Ecology **82**:1584-1596.

Lord, C., C. Brun, M. Hautecoeur, and P. Keith. 2010. Insights on endemism: comparison of the duration of the marine larval phase estimated by otolith microstructural analysis of three amphidromous *Sicyopterus* species (Gobioidei: Sicydiinae) from Vanuatu and New Caledonia. Ecology of Freshwater Fish **19**:26-38.

Lord, C., F. Morat, R. Lecomte-Finiger, and P. Keith. 2012. Otolith shape analysis for three *Sicyopterus* (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and Vanuatu. Environmental Biology of Fishes **93**:209-222.

Marco-Rius, F., P. Caballero, P. Moran, and C. Garcia de Leaniz. 2013. Mixed-effects modelling of scale growth profiles predicts the occurrence of early and late fish migrants. Plos One **8**:e61744.

Marco-Rius, F., P. Caballero, P. Morán, and C. Garcia de Leaniz. 2012. And the last shall be first: heterochrony and compensatory marine growth in sea trout (*Salmo trutta*). Plos One **7**:e45528.

Marschall, E. A., T. P. Quinn, D. A. Roff, J. A. Hutchings, N. B. Metcalfe, T. A. Bakke, R. L. Saunders, and N. L. Poff. 1998. A framework for understanding Atlantic salmon (*Salmo salar*) life history. Canadian Journal of Fisheries and Aquatic Sciences **55**:48-58.

Marteinsdottir, G., B. Gunnarsson, and I. M. Suthers. 2000. Spatial variation in hatch date distributions and origin of pelagic juvenile cod in Icelandic waters. ICES Journal of Marine Science **57**:1182-1195.

McCormick, S. D., L. P. Hansen, T. P. Quinn, and R. L. Saunders. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences **55**:77-92.

McDowall, R. M. 1968. *Galaxias maculatus* (Jenyns), the New Zealand Whitebait. New Zealand Marine Department, Fisheries Research Bulletin **2**:1-83.

McDowall, R. M. 1988. Diadromy in fishes: Migrations between freshwater and marine environments. Croom Helm, London.

McDowall, R. M. 1990. New Zealand freshwater fishes: a natural history and guide. Heinemann Reed, Auckland.

McDowall, R. M. 1991. Conservation and management of the whitebait fishery. Department of Conservation Science and Research Series **38**:1-18.

McDowall, R. M. 1995. Seasonal pulses in migrations of New Zealand diadromous fish and the potential impacts of river mouth closure. New Zealand Journal of Marine and Freshwater Research **29**:517-526.

McDowall, R. M. 1996. Managing the New Zealand whitebait fishery: a critical review of the role and performance of the Department of Conservation. National lnstitute of Water and Atmospheric Research Ltd., Christchurch, New Zealand.

McDowall, R. M. 1998. Driven by diadromy: its role in the historical and ecological biogeography of the New Zealand freshwater fish fauna. Italian Journal of Zoology **65**:73-85.

McDowall, R. M. 1999a. Migration season of whitebait of giant kokopu, *Galaxias argenteus*., Department of Conservation, Wellington.

McDowall, R. M. 1999b. West Coast whitebait fishing closed areas workshop. Department of Conservation, Wellington.

McDowall, R. M. 2003. Variation in vertebral number in galaxiid fishes (Teleostei : Galaxiidae): a legacy of life history, latitude and length. Environmental Biology of Fishes **66**:361-381.

McDowall, R. M. 2007. On amphidromy, a distinct form of diadromy in aquatic organisms. Fish and Fisheries **8**:1-13.

McDowall, R. M. 2009. Early hatch: a strategy for safe downstream larval transport in amphidromous gobies. Reviews in Fish Biology and Fisheries **19**:1-8.

McDowall, R. M. 2010. Why be amphidromous: expatrial dispersal and the place of source and sink population dynamics? Reviews in Fish Biology and Fisheries **20**:87-100.

McDowall, R. M., and S. C. Charteris. 2006. The possible adaptive advantages of terrestrial egg deposition in some fluvial diadromous galaxiid fishes (Teleostei : Galaxiidae). Fish and Fisheries **7**:153-164.

McDowall, R. M., and G. A. Eldon. 1980. The ecology of whitebait migrations (Galaxiidae: *Galaxias* spp.). New Zealand Ministry of Agriculture and Fisheries, Fisheries Research Bulletin **20**:1-172.

McDowall, R. M., and G. R. Kelly. 1999. Date and age at migration in juvenile giant kokopu, *Galaxias argenteus* (Gmelin) (Teleostei : Galaxiidae) and estimation of spawning season. New Zealand Journal of Marine and Freshwater Research **33**:263-270.

McDowall, R. M., C. P. Mitchell, and E. B. Brothers. 1994. Age at migration from the sea of juvenile *Galaxias* in New Zealand (Pisces, Galaxiidae). Bulletin of Marine Science **54**:385-402.

McDowall, R. M., D. A. Robertson, and R. Saito. 1975. Occurrence of Galaxiid larvae and juveniles in the sea. New Zealand Journal of Marine and Freshwater Research **9**:1-9.

McLeod, I. M., M. I. McCormick, P. L. Munday, T. D. Clark, A. S. Wenger, R. M. Brooker, M. Takahashi, and G. P. Jones. 2015. Latitudinal variation in larval development of

coral reef fishes: implications of a warming ocean. Marine Ecology Progress Series **521**:129-141.

Meekan, M. G., and L. Fortier. 1996. Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. Marine Ecology Progress Series **137**:25-37.

Menge, B. A., J. Lubchenco, M. E. S. Bracken, F. Chan, M. M. Foley, T. L. Freidenburg, S. D. Gaines, G. Hudson, C. Krenz, H. Leslie, D. N. L. Menge, R. Russell, and M. S. Webster. 2003. Coastal oceanography sets the pace of rocky intertidal community dynamics. Proceedings of the National Academy of Sciences of the United States of America **100**:12229-12234.

Metcalfe, N. B., and J. E. Thorpe. 1990. Determinants of geographical variation in the age of seaward-migrating salmon, *Salmo salar*. Journal of Animal Ecology **59**:135-145.

Metcalfe, N. B., and J. E. Thorpe. 1992. Early predictors of life-history events: the link between first feeding date, dominance and seaward migration in Atlantic salmon, *Salmo salar* L. Journal of Fish Biology **41**:93-99.

Midway, S. R., S. X. Cadrin, and F. S. Scharf. 2014. Southern flounder (*Paralichthys lethostigma*) stock structure inferred from otolith shape analysis. Fishery Bulletin **112**:326-338.

Midway, S. R., T. Wagner, S. A. Arnott, P. Biondo, F. Martinez-Andrade, and T. F. Wadsworth. 2015. Spatial and temporal variability in growth of southern flounder (*Paralichthys lethostigma*). Fisheries Research **167**:323-332.

Milano, D., P. H. Vigliano, and D. A. Beauchamp. 2016. Effect of body size and temperature on respiration of *Galaxias maculatus* (Pisces: Galaxiidae). New Zealand Journal of Marine and Freshwater Research:1-9.

Ministry for the Environment. 2010. River environment classification New Zealand. Wellington, New Zealand.

Mitchell, C. P. 1991. Deposition of *Galaxias fasciatus* eggs with *Galaxias maculatus* eggs at a tidal site. New Zealand Journal of Marine and Freshwater Research **25**:201-205.

Morgan, M. J., and E. B. Colbourne. 1999. Variation in maturity-at-age and size in three populations of American plaice. ICES Journal of Marine Science **56**:673-688.

Morrongiello, J. R., and R. E. Thresher. 2015. A statistical framework to explore ontogenetic growth variation among individuals and populations: a marine fish example. Ecological Monographs **85**:93-115.

Morrongiello, J. R., C. T. Walsh, C. A. Gray, J. R. Stocks, and D. A. Crook. 2014. Environmental change drives long-term recruitment and growth variation in an estuarine fish. Global Change Biology **20**:1844-1860.

Murphy, C. A., and J. Cowan. 2007. Production, marine larval retention or dispersal, and recruitment of amphidromous Hawaiian gobioids: issues and implications. Biology of Hawaiian Streams and Estuaries **3**:63-74.

Murphy, R. J., M. H. Pinkerton, K. M. Richardson, J. M. Bradford-Grieve, and P. W. Boyd. 2001. Phytoplankton distributions around New Zealand derived from SeaWiFS remotely-sensed ocean colour data. New Zealand Journal of Marine and Freshwater Research **35**:343-362.

Myers, G. S. 1949. Usage of anadromous, catadromous and allied terms for migratory fishes. Copeia:89-97.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution **4**:133-142.

Nanninga, G. B., and M. L. Berumen. 2014. The role of individual variation in marine larval dispersal. Frontiers in Marine Science **1**:1-17.

Nichols, K. M., A. F. Edo, P. A. Wheeler, and G. H. Thorgaard. 2008. The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. Genetics **179**:1559-1575.

O'Connor, M. I., J. F. Bruno, S. D. Gaines, B. S. Halpern, S. E. Lester, B. P. Kinlan, and J. M. Weiss. 2007. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proceedings of the National Academy of Sciences of the United States of America **104**:1266-1271.

Ohms, H. A., M. R. Sloat, G. H. Reeves, C. E. Jordan, and J. B. Dunham. 2014. Influence of sex, migration distance, and latitude on life history expression in steelhead and rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences **71**:70-80.

Opdal, A. F., F. B. Vikebo, and O. Fiksen. 2011. Parental migration, climate and thermal exposure of larvae: spawning in southern regions gives Northeast Arctic cod a warm start. Marine Ecology Progress Series **439**:255-262.

Oresland, V., and C. Andre. 2008. Larval group differentiation in Atlantic cod (*Gadus morhua*) inside and outside the Gullmar Fjord. Fisheries Research **90**:9-16.

Otero, J., J. H. L'Abee-Lund, T. Castro-Santos, K. Leonardsson, G. O. Storvik, B. Jonsson, B. Dempson, I. C. Russell, A. J. Jensen, J. L. Bagliniere, M. Dionne, J. D. Armstrong, A. Romakkaniemi, B. H. Letcher, J. F. Kocik, J. Erkinaro, R. Poole, G. Rogan, H. Lundqvist, J. C. MacLean, E. Jokikokko, J. V. Arnekleiv, R. J. Kennedy, E. Niemela, P. Caballero, P. A. Music, T. Antonsson, S. Gudjonsson, A. E. Veselov, A. Lamberg, S. Groom, B. H. Taylor, M. Taberner, M. Dillane, F. Arnason, G. Horton, N. A. Hvidsten, I. R. Jonsson, N. Jonsson, S. McKelvey, T. F. Naesje, O. Skaala, G. W. Smith, H. Saegrov, N. C. Stenseth, and L. A. Vollestad. 2014. Basin-scale phenology and effects of climate variability on global timing of initial seaward migration of Atlantic salmon *(Salmo salar*). Global Change Biology **20**:61-75.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science **173**:1124-1127.

Paul, K., R. Oeberst, and C. Hammer. 2013. Evaluation of otolith shape analysis as a tool for discriminating adults of Baltic cod stocks. Journal of Applied Ichthyology **29**:743-750.

Pepin, P., J. F. Dower, and H. P. Benoît. 2001. The role of measurement error on the interpretation of otolith increment width in the study of growth in larval fish. Canadian Journal of Fisheries and Aquatic Sciences **58**:2204-2212.

Pepin, P., G. T. Evans, and T. H. Shears. 1999. Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field. ICES Journal of Marine Science **56**:697-706.

Quinn, J. M., and C. W. Hickey. 1990. Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. New Zealand Journal of Marine and Freshwater Research **24**:387-409.

R Core Development Team. 2015. R: A language and environment for statistical computing R Foundation for Statistical Computing Vienna, Austria.

Radtke, R. L., R. A. Kinzie, and D. J. Shafer. 2001. Temporal and spatial variation in length of larval life and size at settlement of the Hawaiian amphidromous goby *Lentipes concolor*. Journal of Fish Biology **59**:928-938.

Richardson, A. J. 2008. In hot water: zooplankton and climate change. ICES Journal of Marine Science **65**:279-295.

Robert, D., P. Pepin, J. F. Dower, and L. Fortier. 2014. Individual growth history of larval Atlantic mackerel is reflected in daily condition indices. ICES Journal of Marine Science **71**:1001-1009.

Roff, D. A. 1996. The evolution of threshold traits in animals. Quarterly Review of Biology **71**:3-35.

Ross, P. M., I. D. Hogg, C. A. Pilditch, and C. J. Lundquist. 2009. Phylogeography of New Zealand's coastal benthos. New Zealand Journal of Marine and Freshwater Research **43**:1009-1027.

Rowe, D. K., and G. Kelly. 2009. Duration of the oceanic phase for inanga whitebait (Galaxiidae) is inversely related to growth rate at sea. Pages 343-354 *in* A. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery, editors. Challenges for diadromous fishes in a dynamic global environment. American Fisheries Society, Halifax, Canada.

Ruggerone, G. T., J. L. Nielsen, and B. A. Agler. 2009. Linking marine and freshwater growth in western Alaska Chinook salmon *Oncorhynchus tshawytscha*. Journal of Fish Biology **75**:1287-1301.

Salinas-de-Leon, P., T. Jones, and J. J. Bell. 2012. Successful determination of larval dispersal distances and subsequent settlement for long-lived pelagic larvae. Plos One **7**: e32788.

Schiel, D. R. 2004. The structure and replenishment of rocky shore intertidal communities and biogeographic comparisons. Journal of Experimental Marine Biology and Ecology **300**:309-342.

Schismenou, E., M. Giannoulaki, K. Tsiaras, E. Lefkaditou, G. Triantafyllou, and S. Somarakis. 2014. Disentangling the effects of inherent otolith growth and model-simulated ecosystem parameters on the daily growth rate of young anchovies. Marine Ecology Progress Series **515**:227-237.

Schismenou, E., M. Palmer, M. Giannoulaki, I. Alvarez, and K. Tsiaras. 2016. Seasonal changes in otolith increment width trajectories and the effect of temperature on the daily growth rate of young sardines. Fisheries Oceanography **25**:362-372.

Schmidt, D. J., D. A. Crook, J. I. Macdonald, J. A. Huey, B. P. Zampatti, S. Chilcott, T. A. Raadik, and J. M. Hughes. 2014. Migration history and stock structure of two putatively diadromous teleost fishes, as determined by genetic and otolith chemistry analyses. Freshwater Science **33**:193-206.

Schmidt, D. J., D. A. Crook, J. P. O'Connor, and J. M. Hughes. 2011. Genetic analysis of threatened Australian grayling *Prototroctes maraena* suggests recruitment to coastal rivers from an unstructured marine larval source population. Journal of Fish Biology **78**:98-111.

Secor, D. H. 2007. The year-class phenomenon and the storage effect in marine fishes. Journal of Sea Research **57**:91-103.

Secor, D. H., and L. A. Kerr. 2009. Lexicon of life cycle diversity in diadromous and other fishes. Pages 537-556 *in* A. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery, editors. Challenges for diadromous fishes in a dynamic global environment. American Fisheries Society, Halifax, Canada.

Semmens, D. 2008. Maternal effects on offspring fitness in an Australian native fish, *Galaxias maculatus*. Ph.D. University of Melbourne, Melbourne, Australia.

Semmens, D., and S. E. Swearer. 2012. Trade-offs obscure the relationship between egg size and larval traits in the diadromous fish *Galaxias maculatus*. Marine Ecology Progress Series **461**:165-174.

Shears, N. T., F. Smith, R. C. Babcock, C. A. J. Duffy, and E. Villouta. 2008. Evaluation of biogeographic classification schemes for conservation planning: Application to New Zealand's coastal marine environment. Conservation Biology **22**:467-481.

Shen, K. N., Y. C. Lee, and W. N. Tzeng. 1998. Use of otolith microchemistry to investigate the life history pattern of gobies in a Taiwanese stream. Zoological Studies **37**:322-329.

Shen, K. N., and W. N. Tzeng. 2002. Formation of a metamorphosis check in otoliths of the amphidromous goby *Sicyopterus japonicus*. Marine Ecology Progress Series **228**:205-211.

Shen, K. N., and W. N. Tzeng. 2008. Reproductive strategy and recruitment dynamics of amphidromous goby *Sicyopterus japonicus* as revealed by otolith microstructure. Journal of Fish Biology **73**:2497-2512.

Shiao, J. C., C. S. Tzeng, P. C. Li, and K. N. I. Bell. 2015. Upstream migration and marine early life history of amphidromous gobies inferred from otolith increments and microchemistry. Environmental Biology of Fishes **98**:933-950.

Shiao, J. C., W. N. Tzeng, A. Collins, and D. J. Jellyman. 2001. Dispersal pattern of glass eel stage of *Anguilla australis* revealed by otolith growth increments. Marine Ecology Progress Series **219**:241-250.

Shima, J. S., and A. M. Findlay. 2002. Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. Marine Ecology Progress Series **235**:303-309.

Shima, J. S., and S. E. Swearer. 2010. The legacy of dispersal: larval experience shapes persistence later in the life of a reef fish. Journal of Animal Ecology **79**:1308-1314.

Shulzitski, K., S. Sponaugle, M. Hauff, K. D. Walter, and R. K. Cowen. 2016. Encounter with mesoscale eddies enhances survival to settlement in larval coral reef fishes. Proceedings of the National Academy of Sciences of the United States of America **113**:6928-6933.

Sim-Smith, C. J., A. G. Jeffs, and C. A. Radford. 2012. Variation in the growth of larval and juvenile snapper, *Chrysophrys auratus* (Sparidae). Marine and Freshwater Research **63**:1231-1243.

Simonin, P. W., D. L. Parrish, L. G. Rudstam, B. Pientka, and P. J. Sullivan. 2016. Interactions between hatch dates, growth rates, and mortality of age-0 native rainbow smelt and nonnative alewife in Lake Champlain. Transactions of the American Fisheries Society **145**:649-656.

Sloat, M. R., D. J. Fraser, J. B. Dunham, J. A. Falke, C. E. Jordan, J. R. McMillan, and H. A. Ohms. 2014. Ecological and evolutionary patterns of freshwater maturation in Pacific and Atlantic salmonines. Reviews in Fish Biology and Fisheries **24**:689-707.

Smith, A. C., and J. S. Shima. 2011. Variation in the effects of larval history on juvenile performance of a temperate reef fish. Austral Ecology **36**:830-838.

Snelder, T. H., and B. J. F. Biggs. 2002. Multiscale river environment classification for water resources management. JAWRA Journal of the American Water Resources Association **38**:1225-1239.

Snelder, T. H., B. J. F. Biggs, and R. A. Woods. 2005. Improved eco-hydrological classification of rivers. River Research and Applications **21**:609-628.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science **60**:1129-1157.

Sorensen, P. W., and K. A. Hobson. 2005. Stable isotope analysis of amphidromous Hawaiian gobies suggests their larvae spend a substantial period of time in freshwater river plumes. Environmental Biology of Fishes **74**:31-42.

Sponaugle, S. 2010. Otolith microstructure reveals ecological and oceanographic processes important to ecosystem-based management. Environmental Biology of Fishes **89**:221-238.

Sponaugle, S., and K. Grorud-Colvert. 2006. Environmental variability, early life-history traits, and survival of new coral reef fish recruits. Integrative and Comparative Biology **46**:623-633.

Sponaugle, S., K. Grorud-Colvert, and D. Pinkard. 2006. Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish *Thalassoma bifasciatum* in the Florida Keys. Marine Ecology Progress Series **308**:1-15.

Starrs, D., B. C. Ebner, and C. J. Fulton. 2016. All in the ears: unlocking the early life history biology and spatial ecology of fishes. Biological Reviews **91**:86-105.

Stearns, S. C. 1976. Life history tactics - review of ideas. Quarterly Review of Biology **51**:3-47.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press Oxford.

Stephens, S. A., N. Broekhuizen, A. B. Macdiarmid, C. J. Lundquist, L. McLeod, and R. Haskew. 2006. Modelling transport of larval New Zealand abalone (*Haliotis iris*) along an open coast. Marine and Freshwater Research **57**:519-532.

Stevens, J. C. B., M. J. H. Hickford, and D. R. Schiel. 2016. Evidence of iteroparity in the widely distributed diadromous fish inanga *Galaxias maculatus* and potential implications for reproductive output. Journal of Fish Biology **89**:1931-1946.

Stransky, C. 2005. Geographic variation of golden redfish *(Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. ICES Journal of Marine Science **62**:1691-1698.

Strauss, R. E. 2010. Discriminating groups of organisms. Pages 73-91 *in* A. M. T. Elewa, editor. Morphometrics for nonmorphometricians. Springer Berlin Heidelberg, Berlin, Heidelberg.

Sutton, P. J. H. 2003. The Southland Current: a subantarctic current. New Zealand Journal of Marine and Freshwater Research **37**:645-652.

Sykes, G. E., C. J. Johnson, and J. M. Shrimpton. 2009. Temperature and flow effects on migration timing of Chinook Salmon smolts. Transactions of the American Fisheries Society **138**:1252-1265.

Tabouret, H., M. Tomadin, L. Taillebois, M. Iida, C. Lord, C. Pecheyran, and P. Keith. 2014. Amphidromy and marine larval phase of ancestral gobioids *Rhyacichthys guilberti* and *Protogobius attiti* (Teleostei: Rhyacichthyidae). Marine and Freshwater Research **65**:776-783.

Taillebois, L., K. Maeda, S. Vigne, and P. Keith. 2012. Pelagic larval duration of three amphidromous Sicydiinae gobies (Teleostei: Gobioidei) including widespread and endemic species. Ecology of Freshwater Fish **21**:552-559.

Taylor, M. J. 2002. The national inanga spawning database: trends and implications for spawning site management. Science for Conservation **188**:1-37.

Teichert, N., M. Richarson, P. Valade, and P. Gaudin. 2012. Reproduction and marine life history of an endemic amphidromous gobiid fish of Réunion Island. Aquatic Biology **15**:225-236.

Teichert, N., P. Valade, A. Fostier, H. Grondin, and P. Gaudin. 2016a. Reproductive biology of an endemic amphidromous goby, *Cotylopus acutipinnis*, from La Réunion Island. Marine and Freshwater Research **67**:526-536.

Teichert, N., P. Valade, A. Fostier, R. Lagarde, and P. Gaudin. 2014. Reproductive biology of an amphidromous goby, *Sicyopterus lagocephalus*, in La Réunion Island. Hydrobiologia **726**:123-141.

Teichert, N., P. Valade, H. Grondin, E. Trichet, F. Sardenne, and P. Gaudin. 2016b. Pelagic larval traits of the amphidromous goby *Sicyopterus lagocephalus* display seasonal variations related to temperature in La Réunion Island. Ecology of Freshwater Fish **25**:234-247.

Theriault, V., and J. J. Dodson. 2003. Body size and the adoption of a migratory tactic in brook charr. Journal of Fish Biology **63**:1144-1159.

Thorrold, S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer, J. E. Neigel, S. G. Morgan, and R. R. Warner. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. Bulletin of Marine Science **70**:291-308.

Thorstad, E. B., C. D. Todd, I. Uglem, P. A. Bjorn, P. G. Gargan, K. W. Vollset, E. Halttunen, S. Kalas, M. Berg, and B. Finstad. 2016. Marine life of the sea trout. Marine Biology **163**:1-19.

Treml, E. A., J. Roberts, P. N. Halpin, H. P. Possingham, and C. Riginos. 2015. The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. Diversity and Distributions **21**:465-476.

Treml, E. A., J. J. Roberts, Y. Chao, P. N. Halpin, H. P. Possingham, and C. Riginos. 2012. Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. Integrative and Comparative Biology **52**:525-537.

Trip, E. D. L., K. D. Clements, D. Raubenheimer, and J. H. Choat. 2014. Temperature-related variation in growth rate, size, maturation and life span in a marine herbivorous fish over a latitudinal gradient. Journal of Animal Ecology **83**:866-875.

Tsukamoto, K., and K. Uchida. 1992. Migration mechanism of the ayu. Pages 145-172 *in* V. I. Ilyichev and V. V. Anikiev, editors. Oceanic and anthropogenic controls of life in the Pacific Ocean: proceedings of the 2nd Pacific symposium on marine sciences, Nadhodka, Russia, August 11–19, 1988. Springer Netherlands, Dordrecht.

Turner, S. M., and K. E. Limburg. 2012. Comparison of juvenile alewife growth and movement in a large and a small watershed. Marine and Coastal Fisheries **4**:337-345.

Turner, S. M., and K. E. Limburg. 2016. Juvenile river herring habitat use and marine emigration trends: comparing populations. Oecologia **180**:77-89.

Underwood, J. N., M. J. Travers, and J. P. Gilmour. 2012. Subtle genetic structure reveals restricted connectivity among populations of a coral reef fish inhabiting remote atolls. Ecology and Evolution **2**:666-679.

Vanhaecke, D., C. G. de Leaniz, G. Gajardo, C. J. Thomas, and S. Consuegra. 2012. Metapopulation dynamics of a diadromous galaxiid fish and potential effects of salmonid aquaculture. Freshwater Biology **57**:1241-1252.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York.

Vigliola, L., and M. G. Meekan. 2002. Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. Oecologia **131**:89-93.

Vincenzi, S., M. Mangel, D. Jesensek, J. C. Garza, and A. J. Crivelli. 2016. Within- and among-population variation in vital rates and population dynamics in a variable environment. Ecological Applications **26**:2086-2102.

Vollestad, L. A., J. Peterson, and T. P. Quinn. 2004. Effects of freshwater and marine growth rates on early maturity in male coho and Chinook salmon. Transactions of the American Fisheries Society **133**:495-503.

Walter, R., J. Hogan, M. Blum, R. Gagne, E. Hain, J. Gilliam, and P. McIntyre. 2012. Climate change and conservation of endemic amphidromous fishes in Hawaiian streams. Endangered Species Research **16**:261-272.

Warburton, M. L. 2015. Migratory movements of torrentfish (C*heimarrichthys fosteri,* Haast 1874). Ph.D. University of Otago, Dunedin, New Zealand.

Ward, R. D. 2000. Genetics in fisheries management. Hydrobiologia **420**:191-201.

Watanabe, S., M. Iida, S. Hagihara, H. Endo, K. Matsuura, and K. Tsukamoto. 2011. First collection of amphidromous goby post-larvae of *Sicyopterus japonicus* in the ocean off Shikoku, Japan. Cybium **35**:371-379.

Waters, J. M., L. H. Dijkstra, and G. P. Wallis. 2000. Biogeography of a southern hemisphere freshwater fish: how important is marine dispersal? Molecular Ecology **9**:1815-1821.

Waters, J. M., and M. S. Roy. 2004. Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? Molecular Ecology **13**:2797-2806.

Way, C. M., A. J. Burky, J. M. Harding, S. Hau, and W. Puleloa. 1998. Reproductive biology of the endemic goby, *Lentipes concolor*, from Makamaka'ole Stream, Maui and Waikolu Stream, Moloka'i. Environmental Biology of Fishes **51**:53-65.

Weber, M. J., B. C. Ruebush, S. M. Creque, R. A. Redman, S. J. Czesny, D. H. Wahl, and J. M. Dettmers. 2015. Early life history of alewife *Alosa pseudoharengus* in southwestern Lake Michigan. Journal of Great Lakes Research **41**:436-447.

Weisberg, S., G. Spangler, and L. S. Richmond. 2010. Mixed effects models for fish growth. Canadian Journal of Fisheries and Aquatic Sciences **67**:269-277.

Wells, B. K., K. D. Friedland, and L. M. Clarke. 2003. Increment patterns in otoliths and scales from mature Atlantic salmon *Salmo salar*. Marine Ecology Progress Series **262**:293-298.

Wenger, A. S., J. Whinney, B. Taylor, and F. Kroon. 2016. The impact of individual and combined abiotic factors on daily otolith growth in a coral reef fish. Scientific Reports **6**:28875.

Winemiller, K. O. 2005. Life history strategies, population regulation, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences **62**:872-885.

Wood, S. N. 2006. Generalised additive models: an introduction with R. Chapman and Hall, London UK.

Wootton, R. J. 1998. Ecology of teleost fishes. 2nd edition. Kluwer Academic Publishers, Dordrecht.

Yungnickel, M. R. 2017. New Zealand's whitebait fishery: spatial and temporal variation in species composition and morphology. MSc. University of Canterbury, Christchurch, New Zealand.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York